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ELAM BARTHOLOMEW

E. T. BARTHOLOMEW [1]

(WITH PORTRAIT)

Elam Bartholomew was born at Strasburg, Pennsylvania, June 9, 1852. He was later taken by his parents to Ohio and then to a farm near Farmington, Illinois, where he completed the district school and grew to early manhood. When Dr. Bartholomew decided to teach school he found that a general knowledge of botany, which he had never studied, was a prerequisite to the obtaining of a teacher's certificate in Illinois. Accordingly, he purchased a copy of *Gray's Lessons in Botany* and had soon passed the examinations in botany as well as in the other required subjects. Following the close of his term of school in the spring of 1874 he decided to "go west." Arriving in northwest Kansas he at once homesteaded a farm in the Bow Creek Valley, near Stockton. He lived on this farm for 55 years. In 1929 he moved to the Fort Hays Kansas State College at Hays, Kansas, where he became curator of the mycological herbarium. He held this position until the time of his death, November 18, 1934.

The subject of this sketch did not attend school except as mentioned in the preceding paragraph, but the purchase of *Gray's Lessons in Botany* in 1873 started him in a line of work that gradually occupied more and more of his time until it became his life work.

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Soon after his arrival in Kansas he began to collect, preserve, and classify flowering plants. By 1885 he had in his herbarium a specimen of every phanerogam growing in that part of the state. As head of a rapidly enlarging family and as a farmer he was very busy, but at every spare moment during the day and until late hours at night, he was always found with a book in his hand. Without any assistance he acquainted himself not only with books on phanerogamic botany but with the Latin that was needed for his scientific work. He tutored several students in the latter subject so that they could pass the entrance examination admitting them to the Kansas State Agricultural College.

One day in July, 1885, Dr. Bartholomew, like Cincinnatus of old, was plowing in his field when W. A. Kellerman, then professor of botany at the Kansas State Agricultural College, came to see him. The two had never met but had corresponded much concerning phanerogamic matters. After some time Dr. Kellerman stooped down, pulled a leaf from a weed, and straightening up said, "Bartholomew, why don't you study something that is really interesting? Look at this leaf." It was an *Amaranthus* leaf and was covered with white pustules (*Albugo*) on the lower surface. Bartholomew did become interested and many times in later years as he developed his mycological herbarium, he was heard to remark: "The plucking of that leaf by Professor Kellerman from a weed in my cornfield marked a turning point in my life."

In 1887 Dr. Bartholomew's mycological herbarium consisted of only 31 labeled specimens, but at the time of his death the number had increased to about 38,000,[2] being composed of approximately 850 Agarics, 1,300 Polypores, 14,000 Rusts, 1,200 Smuts, 7,450 miscellaneous saprophytes, and 13,000 other forms. In addition to North American fungi the herbarium contains many hundreds of specimens from Sydow's Fungi Exotici, Phycomycetes, Mycotheca Germanica, Uredineen, and Ustilagineen; Petrak's Czechoslovakian fungi; Vestergren's Swedish fungi; Fungi Europaei; Cuba, The Philippines, Malaya, and from many other foreign sources.

[2] Figures are for 1931. Since that time the number has been increased to about 40,000.

In 1901, Dr. Bartholomew became editor and publisher of Ellis and Everhart's Fungi Columbiani, composed of specimens of North American fungi of all kinds. He continued this publication until 1917, having published 36 centuries of this work during the years from 1901 to 1917. In 1911 he began the publication of a new work called North American Uredinales. This was somewhat similar to the preceding but included only the rust fungi of North America. He published 35 centuries of this new work. The publication of Fungi Columbiani and North American Uredinales entailed the identification, putting into packets, labeling, and indexing of 427,000 specimens.

On his collecting trips, which took him into every State in the Union, as well as into Canada and Mexico, Dr. Bartholomew personally collected 290,672 specimens which were later identified and appropriately cared for. On his journeys he discovered approximately 470 species of fungi that were new to science. In the work of identifying specimens and the naming of new species he was always closely in touch, either in person or by correspondence, with such men as J. B. Ellis, Charles H. Peck, E. W. D. Holway, J. C. Arthur, Ellsworth Bethel, John Dearness, and many others. He had the pleasure and the inspiration of making collecting trips with all of the men whose names have been mentioned, and with a host of others.

Dr. Bartholomew was always in close touch with much of the botanical work done by the United States Department of Agriculture. From 1891 to 1893 he was engaged by W. T. Swingle, of the Department of Agriculture, to conduct on his farm, which had become the garden spot of that part of Kansas, a series of tests relative to the control or eradication of grain rusts by spraying or by soil treatments. Accounts of some of the results of this work may be found in the Department Year Book for 1892 and in the Journal of Mycology for 1893. Again, under the direction of the U. S. Department of Agriculture, from 1908 to 1913, Dr. Bartholomew conducted an extensive series of tests on the growing of various promising types and strains of corn, cotton, and alfalfa from many different parts of the world. Over 100 different species or types and strains of alfalfa were growing on his farm at one time.

Dr. Bartholomew had several invitations to become connected with colleges and universities, but he always refused until in 1929 when he became curator of the mycological herbarium at the Fort Hays Kansas State College. He gave as his reason for refusal that he preferred to work in private on the farm which he homesteaded in the waning days of the buffalo and antelope; the farm where he had planted hundreds of shade and fruit trees. Although he was reluctant to connect himself with one of the institutions of higher learning, he felt highly honored when in 1898 the Kansas State Agricultural College conferred upon him the honorary degree of Master of Science, and again in 1927 when the same institution conferred upon him the honorary degree of Doctor of Science.

Dr. Bartholomew published few scientific articles. Many might have been written concerning new species which he discovered, criticisms of species already named, host and parasite distribution, and the alternate hosts of certain parasitic fungi, all of which interested him greatly. His correspondence is rich in such things, but he left this work for others to do. In 1899 he published " The Plant Rusts of Kansas " in the Kansas Academy of Science. In 1927 he published " The Fungus Flora of Kansas " as Contribution No. 268 of the Kansas State Agricultural College. This publication lists 1,829 species as having been found in Kansas. Almost 20 per cent (360) of these were new to science. Up to the time of this report only about 465 species had been listed as having been found in Kansas. The great increase was due almost entirely to the work of Dr. Bartholomew. In recommending to the Dean that the manuscript for " The Fungus Flora of Kansas " be published, Dr. L. E. Melchers said, in part: " Dr. Bartholomew is the country's foremost collector of fungi and a student of mycology. . . . It is largely due to Dr. Bartholomew's research efforts that Kansas ranks so high in its report of so large a number of species of fungi." Fifty-eight additional species were reported for Kansas by Dr. Bartholomew in the " Trans. Kansas Acad. Sci." **33**: 82–83. 1930. He personally collected all of these but three.

Dr. Bartholomew's most extensive work was published in book form in 1927 and was entitled " Handbook of the North American

Uredinales." This book included the names and synonyms of all of the plant rusts that had been found up to that time in North America, Greenland, and the West Indies. The list contains 1,240 species and 3,505 synonyms. A revised edition of this book was published in 1933.

Dr. Bartholomew was a member of the American Association for the Advancement of Science, Kansas Academy of Science, American Forestry Association, Mycological Society of America, American Phytopathological Society, and the Delta Upsilon honorary society.

The terms neat, methodical, and accurate characterize the work of Dr. Bartholomew. In collecting and putting up of specimens, "Good enough is not good enough," was an expression he often used. He did not measure success in terms of speed of accomplishment but in terms of reality and completeness. These traits, coupled with his seemingly inexhaustible supply of energy and zeal for accomplishment, perhaps explain how he could remain a successful farmer during the earlier years of his mycological career, and how, in spite of the fact that he was far removed from all of the leading educational and scientific centers, he became a world figure in the field of mycology. Another indication that he was a hard and methodical worker is shown by the fact that in spite of his many other arduous duties, he kept a well-written diary for 19,000 consecutive days (52 years), and it was from this diary that most of the information in this article was taken. A life sketch of Dr. Bartholomew would not be complete without stating that he was interested in and took part in civic and educational matters to the fullest extent of his ability. He would also wish to have a tribute paid to the one who so faithfully encouraged and assisted him in all types of work with which he was concerned, his wife, who survives him, Rachel Isabel Bartholomew.

A NEW PUFFBALL

Elizabeth Eaton Morse

(with plates 12–15)

The Gasteromycete described in this article appears to be the most abundant and widely distributed puffball at high altitudes in the western states. In the herbarium of the University of California specimens of this species had been confused with *Calvatia sculpta* (Hark.) Lloyd,[1] and we had no suspicion that they might be a different species until an inquiry accompanied by a specimen arrived from Doctor P. F. Shope (1933) concerning a Rocky Mountain puffball which has an excessively branched capillitial thread. Prompted by his inquiry, we examined all our material labeled *Calvatia sculpta*, and found that over one-half of it had the same peculiar capillitium as the Colorado plant. During nine years, the writer has collected in many different localities many examples exhibiting the peculiarity mentioned as well as other characters in common.

This puffball has a peridium which suggests *Calvatia caelata, C. sculpta, Scleroderma flavidum,* and *S. aurantium;* a deep, sterile, rooting base which suggests *Bovistella*, and a discrete, excessively branched thread which suggests *Bovista, Bovistella* and *Mycenastrum*. But it cannot be a *Calvatia* because of its much branched, entangled capillitium; it cannot be a *Scleroderma*,[2] because *Scleroderma* has a single layered peridium and a scanty, fragmented capillitium; it cannot be *Bovistella*, because it does not have an apical mouth; it cannot be *Bovista* because it has a sterile base, is not a "tumbler," and has no apical mouth; it cannot be

[1] Lloyd, Myc. Writ. **1**: 203. 1904; Setchell, Sierra Cl. Bull. **6**: 39. 1906; Setchell, Bull. Torrey Club **35**: 291. 1908; Morse, Sierra Cl. Bull. **14**: 61. 1929; Morse, Nat. Mag. June 1931.

[2] We find in Sacc. Syll. **7**: 140. 1888, *Scleroderma fragile* (Lév.) De Toni (*Mycenastrum fragile* Lév.); *S. fragile* has a "nude" peridium and no columella. Patouillard has *Mycenastrum martinicense* with peridium broken up like our plant, but it has a central columella. Our puffball does not fit any of these descriptions. Bull. Soc. Myc. Fr. **18**: 178. 1902.

Mycenastrum, because it does not dehisce in a stellate manner, and it is deeply rooted in soil. Externally, it closely resembles *C. sculpta*—in fact it has always been supposed to be *C. sculpta,* as previously stated, but is very distinct in the character of the capillitium and of the spores. Structurally, it has some characters of both *Bovistella* and *Mycenastrum.* In other words, we have here a puffball which bridges the gap between two large, distinct groups of puffballs: it has characters which, on the one hand, look to the *Lycoperdon* and *Calvatia* group, and, on the other hand, to the *Bovista, Bovistella* and *Mycenastrum* group.

With respect to the correct classification of this puffball we have conferred with mycologists on both sides of the Atlantic and have received several different opinions. We are concurring with the view of Professor W. C. Coker when he says: ". . . from the evidence before me I cannot find that your puffball has ever been described. It seems to me that it is somewhere between *Calvatia* and *Bovistella,* and in reality does not belong to any genus as at present defined. I agree with you that it is not a *Mycenastrum.*"

A rich collection was recently made at Soda Springs, California, from which ample material was secured for completing studies of the early stages of development.

Calbovista gen. nov.

Sporophore medium to large, cremaceous, top-shaped, solid base ending in soil-embedded rhizomorphs. Peridium two-layered: the outer thick, coriaceous and broken into pyramidal plates which fall away from top downward at maturity; the inner layer a delicate membrane. Gleba fragile, dark umber at maturity; subgleba present, distinct, deep. Capillitium abundant, discrete, ochraceous yellow, antler-like. Basidia four-spored.

Calbovista subsculpta sp. nov.

Sporophore cremaceous, irregularly top-shaped, averaging 8 (16) cm. wide by 9 cm. deep, often plicate and contracted towards the rooting base; peridium [3] two-layered, the outer thick, cori-

[3] The great variation in size of mature plants of both *Calvatia sculpta* and *Calbovista subsculpta* may be accounted for by their varied habitats. It should be borne in mind that these puffballs often grow where the snowfall is exceedingly deep, and consequently when the melting season arrives the supply of moisture is continuous for several weeks. Other conditions re-

aceous, broken into irregular three to six sided, low pyramids—usually blunt, sometimes pointed; pyramids 5–8 mm. thick on top of sporophore, gradually becoming shorter on sides, peridium quite thin towards the base. Pyramids show parallel markings [4] on their sides, similar to those found in *Calvatia sculpta.* Inner peridium, an extremely thin, shiny tissue, depressed into areas by the heavy pyramidal plates; sterile base, one-fourth to one-third of the gleba consisting of chambers of moderate size, persisting after fertile tissues are dispersed and becoming more or less purplish with weathering; diaphragm absent; fertile tissues, in young plants not readily distinguished from sterile, pass through color changes when maturing—white to sulphur yellow, to golden brown or mummy brown (Ridgway), to dark umber, always darker than *C. sculpta;* deliquescence of gleba free but never complete.

Microscopic characters.—Glebal chambers closely adjacent, lined with clavate basidia, 10–12.5 μ long by 5–7.5 μ wide, each basidium bearing four spores; sterigmata usually short, 1.7–5.5 μ, the longest set 7.5 μ, length uniform on each basidium. Spores globose, 3–5 μ, ochraceous brown, smooth to faintly warted, uniguttulate, with a hyaline pedicel up to 2.5 μ long; epispore a half micron thick. Capillitium free, consisting of short, discrete units with abundant antler-like branching, much entangled; secondary branches bluntly pointed, not varying much in width from main branch; threads 5–10 μ wide, wall thick up to 2.5 μ, becoming thinner towards the tips; not septate,[5] not pitted, ochraceous yellow, concolorous.

HABITAT. In disintegrated rock mixed with soil or in open coniferous forest, 3,000–11,000 feet above sea level; may be associated with *Calvatia sculpta,* but not exactly in the same colony; a colony of each species at Soda Springs about fifty yards apart.

HABIT. Gregarious, usually single, but occasionally caespitose.

SEASON. Species collected April to August.

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maining favorable, both these species may continue to grow for many days; the largest weight recorded is of *C. sculpta*—four and one-half pounds, General Grant National Park (Roberts). The spiny covering of young plants is often disproportionately thick.

[4] These markings are probably attributable to the variation in temperature of night and day (up to 40° F.); plants do grow during the cold nights, but growth is slower than in the warm daytime and the change of rate leaves a record on the sides of the pyramids.

[5] Threads of *Bovista* and *Bovistella* also not septate in the species examined by Cunningham. Proc. Linn. Soc. of N. S. W. **50**: 368. 1925.

Type collection and locality. Description composite, based on a large collection taken by the author at Soda Springs, California, elevation 6,767 feet, May 7–May 23, 1934. Plants studied and regarded as typical are deposited in the herbarium of the University of California as no. *525436*.

DISTRIBUTION. Colorado: Boulder (Shope).

Idaho: Moscow (Diettert).

Washington: Mount Rainier, Paradise and eastern area (Brockman, Diettert).

California: Mount Shasta City (Whiteley, Morse); Mount Shasta, below Medicine Lake (Morse); Drakesbad and Mineral, Mount Lassen National Park (Morse); Chester, Lassen region (Martin, Gay, Morse); Battle Creek Meadow (Jepson); Quincy, Plumas Co. (Burdick); Merrimac, Butte Co. (Norman); Cisco, Placer Co. (Gould, Cree); Soda Springs, near pass over the Sierras above Truckee and Donner Lake (Jones, Lemon, Saunders, Barnes, Morse); Alpine Lake, Pine Crest, Dardanelles (Morse); Calaveras Big Trees (Wirt); Stanislaus National Forest (Patty); Eagle Meadow, Tuolumne Co. (Grant, 1915); Yosemite National Park (Harwell, Morse); Huntington Lake (Pierson); Rock Creek, east side of Sierras, below Mono Pass, above Bishop (Matthews); General Grant National Park (Roberts, Cunningham, Morse); Sequoia National Park (Dixon, Bracelin, Forster, Morse); Big Pines Park, Los Angeles Co., 7,000 feet elevation (Templeton); Bluff Lake, San Bernardino Co. (Nicholson, 1920).

The genus name proposed suggests the genus *Calvatia* to which it is closely allied and, incidentally, California, where the largest collections have been made; " bovista " suggests both *Bovista* and *Bovistella*, which also have discrete, branched capillitial threads. The species name suggests *C. sculpta*, whose peridium it simulates, and for which it has been mistaken during many years. Discussion and criticism of the opinions and statements herein presented will be welcomed.

I wish to make grateful acknowledgment to Doctor P. F. Shope, University of Colorado; Doctor Carleton Rea, England; Doctor John Dearness, Canada; Doctor W. H. Long, New Mexico; Doctor C. L. Shear, Washington, D. C.; Doctor W. C. Coker and Miss Alma Holland, University of North Carolina; Doctor Lee

Bonar, University of California; Miss Vera P. Mentzer, for assistance in the microscopic studies; also, the numerous and interested collectors of abundant material.

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UNIVERSITY OF CALIFORNIA,
BERKELEY, October 1, 1934

EXPLANATION OF PLATES

Photographs and Photomicrographs by W. C. Matthews

PLATE 12

Calbovista subsculpta: Left, Section of young sporophore; gleba white; rhizomorph solid. Yosemite; Center, Sporophore globose 9 cm.; peridium broken into plates with appressed pyramids, having parallel side markings. Below Yosemite Falls; collected by the author; Right, Peridial plates small, pyramids elevated, tips connivent. Moscow, Idaho; collected by Diettert.

PLATE 13

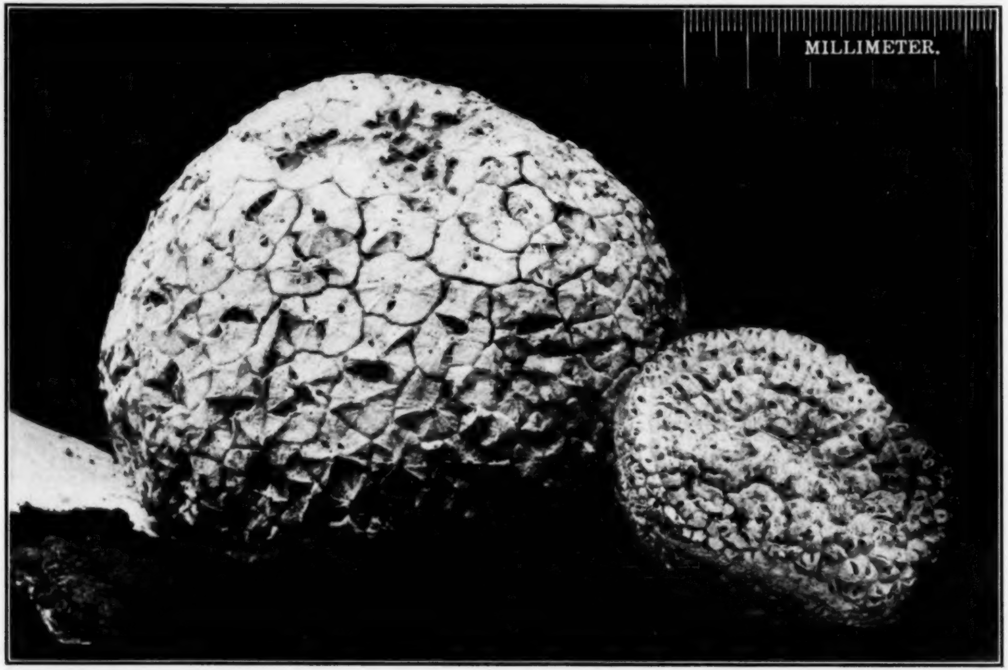
Figs. 1–6 inc. *Calbovista subsculpta:* 1, Sporophore large, flattened globose; gleba depressed into shallow discs by heavy plates, the discs lined by delicate endoperidium which soon disintegrates and merges with glebal tissues. Solid, anastomosed rhizomorphs of hyphae and agglutinated sand. (Grew on the bank of a creek, and when spread out were large enough to fill an ordinary cigar box.) Sequoia National Park; collected by Forster; 2, Young sporophores, perfectly white; peridia cracked. Idaho; collected by Diettert; 3, Vertical, median section; deep sterile subgleba; gleba white; 4, Young stage; peridium cracked. Camp Curry, Yosemite; collected by the author, 1926; 5, 6, Caespitose. Soda Springs, near summit of Sierra; collected by Saunders and Lemon, 1934.

PLATE 14

Figs. 1–5 inc. *Calbovista subsculpta:* 1, Peridial plates large, deep, heavy. Soda Springs; collected by Saunders; 2. Sterile base, dark umber, tinged purplish, embedded in soil; persists a long time. Exoperidium cracking off; endoperidium intact. Yosemite; collected by author, 1926; 3, Section from a Boulder, Colorado plant; peridium and glebal tissues characteristic; collected by Shope; 4, Subgleba deep; gleba beginning to turn dark. Sequoia National Park; collected by Dixon; 5, Reverse of fig. 4, pl. 13.

PLATE 15

Photomicrographs of capillitial threads and spores. × 383. A, *Mycenastrum Corium;* B, *Calbovista subsculpta;* C, *Bovistella radicata;* D, *Calvatia sculpta.*



CALBOVISTA SUBSCULPTA

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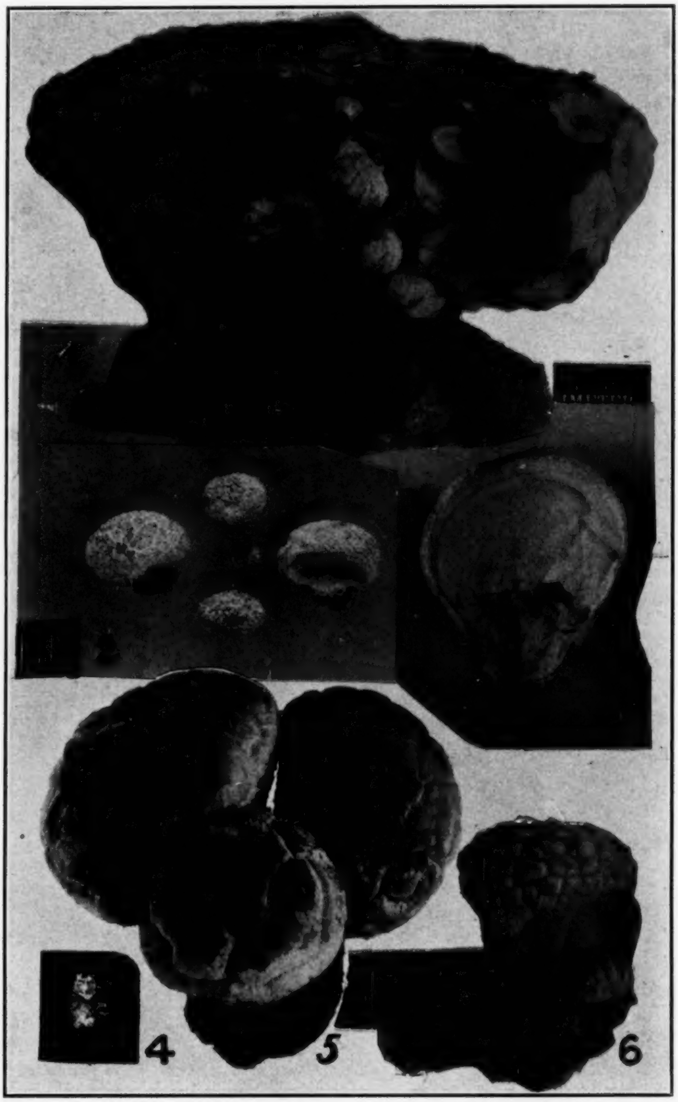
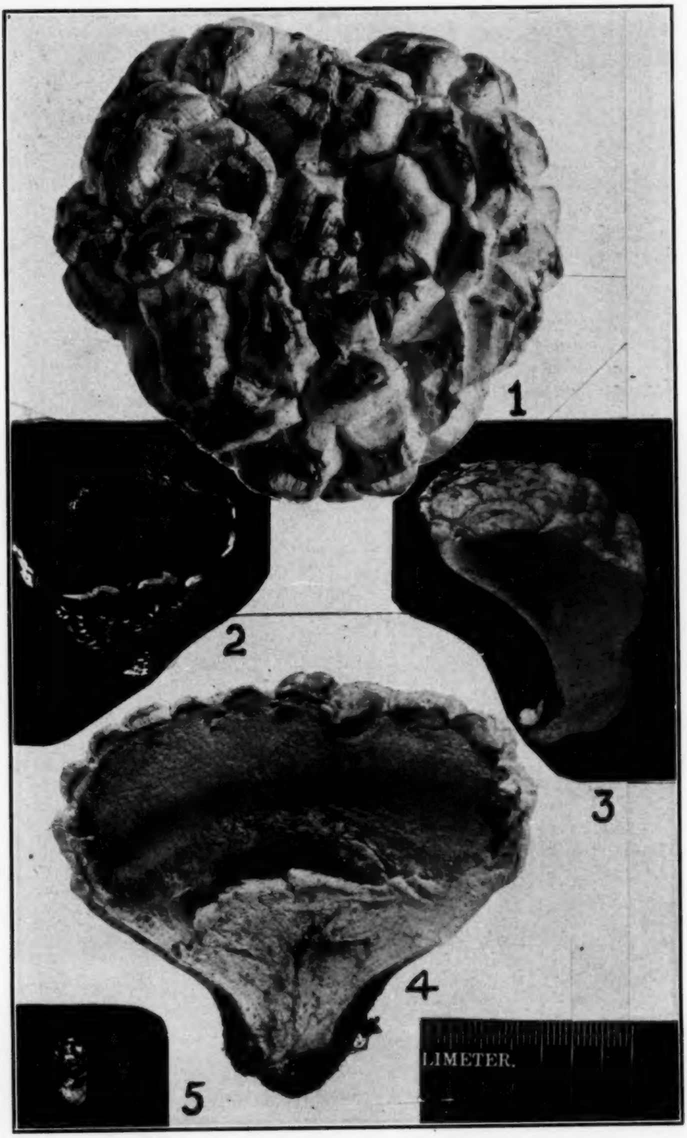


CALBOVISTA SUBSCULPTA

My



Calbovista subsculpta

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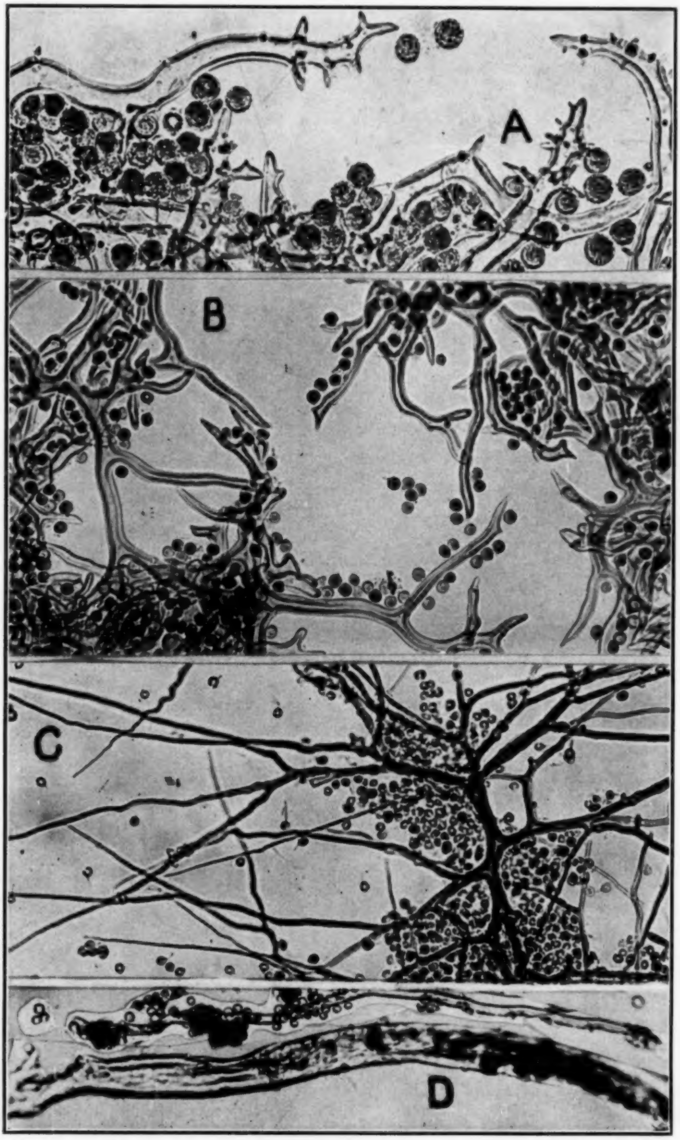

A, MYCENASTRUM CORIUM. B, CALBOVISTA SUBSCULPTA.
C, BOVISTELLA RADICATA. D, CALVATIA SCULPTA.

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Compared:

1. All threads in A, B, C, D alike in one character, being tubular.
2. Threads in A, B, C discrete, i.e. entirely free from peridia. In D long, unbranched or rarely branched, growing from sterile base and endoperidium.
3. Branches in A short, stubby spines, limited to tips.
4. Branches in B antler-like, not much narrower than main trunks, abundant, much entangled.
5. Branches in C numerous on main trunks, elongated to slender, needle-like tips.
6. Spores in B, C, D small, fairly smooth, pedicellate. Spores in A large, very rough.

The capillitial thread in Gasteromycetes is of great taxonomic importance. The thread in the new puffball is very distinct from that in each of the other three species shown in this plate.

STUDIES ON ASCOIDEA RUBESCENS—II CYTOLOGICAL OBSERVATIONS[1]

Leva B. Walker

(with 78 text figures)

That *Ascoidea* shows characteristics of both Phycomycetes and Ascomycetes was pointed out by earlier workers, as Brefeld (**2**), Popta (**11**), and Holtermann (**7**). How strikingly this is true is attested by the fact that Lohwag, 1926 (**8**), concluded that *Ascoidea* is in every respect a Phycomycete while Varitchak, 1928 (**12**) and 1931 (**13**), considered it a simple Ascomycete. The fact that these two recent workers have arrived at such opposite conclusions makes the publication of these observations, largely completed before the publication of Varitchak's detailed paper, seem worth while.

Interest has naturally centered around the development of the spore sac[2] but the nuclear behavior in all stages of development has been studied.

MATERIALS[3]

These studies are based upon materials collected at Ithaca, N. Y., in 1927 and at Lincoln, Nebr., in June, 1930, 1931, 1932, and 1933, and materials grown by the writer as previously described (**14**). Of the fixing fluids used, a dilute formal-acetic-alcohol (neutral formalin, 6 cc.; acetic acid, 1 cc.; and 50 per cent alcohol,

[1] Contribution from the Department of Botany, University of Nebraska, no. 90. I. History and Development. Mycologia **23**: 51–76, *fig. 1–74.* 1931.

[2] In this paper the terms spore sac and spore are substituted for sporangium and sporangiospore used by Brefeld and by the writer in an earlier paper.

[3] In spite of the fact that *Ascoidea* is not well known in this country it seems to be widely distributed and very abundant when moist, warm, but not hot, weather prevails. Around Lincoln, Nebraska, it is commonly present on elms, both in woods and along streets, but disappears entirely after a day with temperatures above 95° F. Besides the first collection at Ithaca, N. Y., and numerous Lincoln collections, materials have also been sent the writer from Iowa City, Iowa, where it grew in a drain pipe, and from Greeley, Colorado, where it occurred on both cottonwood and elm.

93 cc.), Allen's modified Bouin's fluid, and Fleming's strong solution, have given the best results (in the order named), while the various mixtures of chrom-acetic have given the poorest. So much mineral matter is included in the fungus mass that pieces of the fungus placed in an acid fixing solution foam as would a piece of lime placed in the solution. For this reason, in later collections, the fixing solutions were repeatedly changed till foaming ceased before leaving for fixation.

The materials collected at Lincoln, because of the much greater abundance of spore sacs and their more definite orientation (FIG. 1), were much better for cytological studies. Fully as many spore

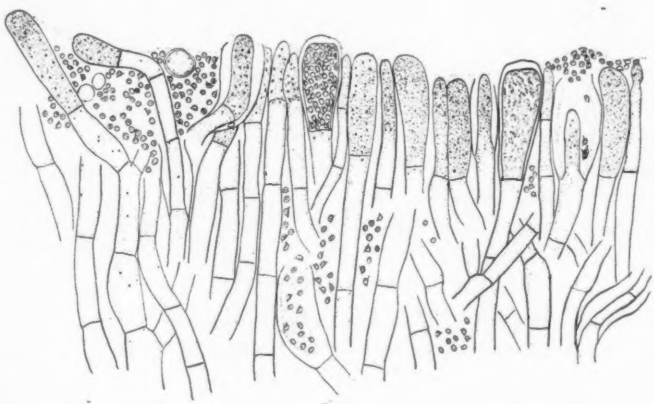

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FIG. 1. A cross section through the peripheral region of a young thallus of *Ascoidea rubescens* to show how reproductive and vegetative cells form a vague hymenium-like layer covering the surface of the thallus. Drawn by aid of camera lucida but telescoped by the omission of hyphal tips in two places.

sacs could be seen on a single slide made from many local collections as would be found on a hundred slides from materials collected at Ithaca. Continued observations on the fungus make it obvious that the collections from Ithaca were old, much depleted, and broken down by the myriads of organisms included in the mass. Materials grown in cultures contained so many depauperate and degenerating spore sacs that little use could be made of them ex-

cept for comparison. So far as could be determined, development was identical in all materials studied.

Serial sections, cut 3–7 μ in thickness, have been used. Sectioning has been very difficult because of hard granules included in the fungus mass. For this reason most of the series were more or less torn and broken. The most satisfactory stains employed were Heidenhain's iron-alum haematoxylin and Fleming's triple stain. The haematoxylin followed by orange G in clove oil gave especially clear differentiation. For staining toto mounts of germinating spores, Brazilin was much better than other stains used. Toto mounts never gave clear differentiation because of the large size of hyphae, spore sacs, and conidia, and the extremely small size of the nuclei. The only stain that gave clear differentiation of the nuclei in toto mounts was secured by using Barrett's smear technique (1 pt. equal pts. of stock iron-alum and haematoxylin, 1 pt. 95 per cent alcohol, 2 pts. glacial acetic acid) but so many crystals usually formed among the hyphae that details were obscured. Also the walls of mature endogenous spores were impermeable to the fluid. With other stains employed it was impossible to secure clear differentiation through unbroken walls.

CELL CONTENT

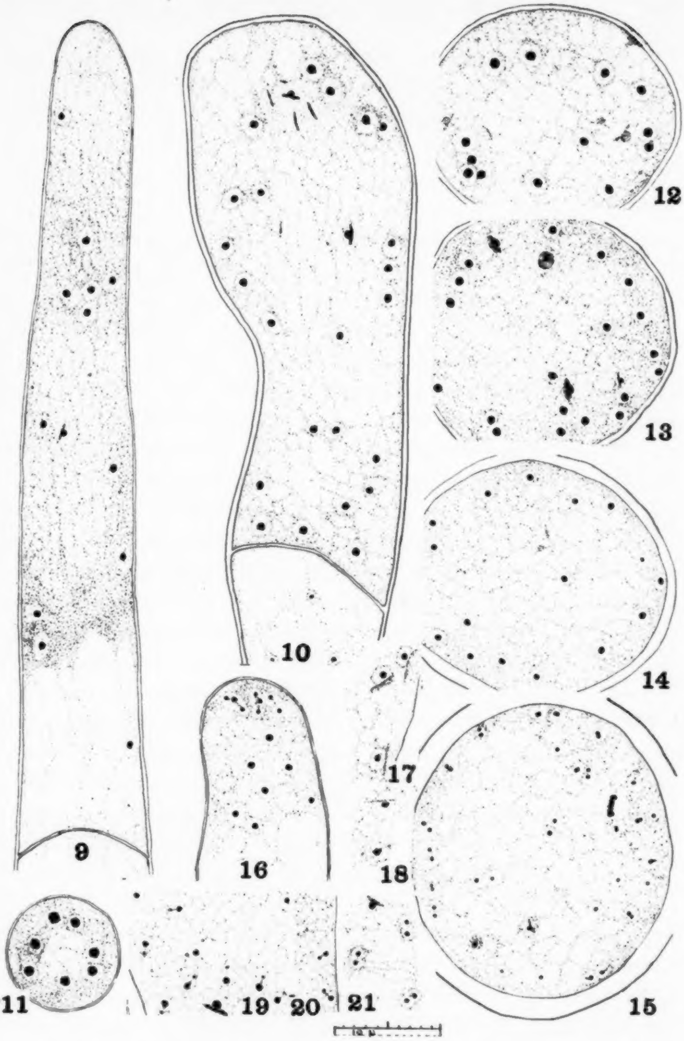
All cells formed by *Ascoidea,* except the spores of the spore sac, are coenocytic. Each nucleus typically contains a relatively large nucleolus and little additional chromatin. The nucleus and especially the nucleolus, are variable in size, being much larger in young cells than in older cells (FIG. 2–15), but structurally they are similar. Many nuclei in hyphae that show other indications of degeneration contain irregular, granular, chromatic masses, such as are shown in spores (FIG. 38, left). Similar nuclei are also occasionally found in vigorously growing hyphae.

Mingled with the nuclei, especially toward the apex of young cells but occurring in all coenocytic cells, are extremely conspicuous, deeply staining bodies of several types. These are most conspicuous in materials fixed with fluids containing osmic acid and stained with haematoxylin but are evident with all fixing fluids and stains. The most conspicuous and definite of these bodies, in surface view, look like densely stained nuclei with extremely large

nucleoli (FIG. 8 AND 13). In side view, however, they appear as deeply stained rods with a homogeneous deeply stained globule attached laterally at the center. Through varying views it is obvious that these bodies consist of a circular disk of granular matter and a globule of homogeneous, highly refractive consistency. These "disk and globule" bodies are usually about the size of the nuclei in the cell, or larger, but in the same cell some may be two or three times as large as others (FIG. 2, 5, 8, 10, 12–15, 19–22, etc.). The origin and identity of these bodies are very perplexing. They are probably degenerating nuclei since what seem to be intermediate stages have been observed. It is possible that nuclei such as shown in figures 17 and 18 represent beginnings of this degeneration. Later stages appear as disks and globules but not so dense. They are most abundantly present in young vigorously growing tips of hyphae developing on old, much elongated hyphae and are much less abundant in tips of young hyphae that have not yet formed many cells. In coenocytic hyphae such as these degenerating nuclei might easily be carried forward in the cytoplasm and this may readily account for their occurrence as observed. Much oil is stored by the fungus and the possibility that they may be structures associated with oil secretion has suggested itself. These bodies are so highly refractive that they can be seen in living hyphae, especially in side view, where the disk appears as a rod-like body. These are undoubtedly the structures that Varitchak (**13**) considers meristematic or sex nuclei. That this is not the case is shown by the facts (1) that they are rarely found surrounded by dense cytoplasm and are usually found in much vacuolate parts of the cell and (2) that they are present at all stages of development except in endogenously developed spores, even occasionally in the slime surrounding developing (FIG. 35 AND 75) and mature spores. The fact that following some fixing solutions they do not stain at all as do nuclei is also a strong reason for considering that they are not nuclei.

Bodies which appear like disks and globules separated from each other also abound, especially in older parts (FIG. 10 AND 35). Various sorts of deeply staining bodies that have a fibrous to crystalline appearance are commonly seen. These are especially

FIGS. 2–8. Details of hyphal tips and young spore sacs to show form and protoplasmic content. 2, a proliferating hyphal tip; 3, a young vegetative tip; 4–6, young spore sacs; 7, an older spore sac on a proliferating hypha; 8, degenerating spore sac.



Figs. 9–15. Nuclear variations. Longitudinal section of a young spore sac, 10, and vegetative cells, 9 and 16, to show similarity of nuclei; 11–15, cross sections of a hyphal tip (11) and spore sacs (12–15) in various stages of development up to the beginning of spore formation to show decrease in nuclear size as related to the thickening of the wall of the spore sac; 17–21, types of nuclei commonly observed, especially in cells with scanty cytoplasm.

frequent during the earlier stages of endogenous spore development (fig. 15, 35, and 36) but may occur in other cells.

MYCELIUM

Except for the hyphal tips, the mycelium is composed of cells with scanty protoplasmic contents. The ultimate cells, on vigorously growing hyphae, are filled with deeply staining protoplasm usually containing from 6 to 20 nuclei. When an apical cell is very young the protoplasm is of quite uniform peripheral density with many small central vacuoles (fig. 3). As the hyphal tip elongates the protoplasm is pushed forward and a large vacuole is formed near the base of the cell (fig. 9). When this basal vacuole has enlarged to about two-thirds of the length of the cell (fig. 1, left of central mature sporangium) a cross wall is formed just below the dense protoplasm in the upper third of the cell. In the penultimate cell the protoplasm is much less dense and is largely peripheral. The protoplasm becomes successively more and more scanty till in old hyphae all active protoplasm seems to have disappeared. Older hyphal cells with dense protoplasmic contents are occasionally found and it is from these that branches develop. Such cells occur often even in old, much broken-down materials and are capable of initiating new growth. In general, hyphal tips destined to continue vegetative growth, maintain approximately the diameter of the hyphae from which they arise (fig. 3), those preparing to form conidia become narrowed at the tip (fig. 9), while those about to form spore sacs widen apically (fig. 4–7). The protoplasmic contents are similar in all. The spore sacs usually develop on hyphae of somewhat larger diameter than those forming only conidia.

Because of apical growth the fungus when growing vigorously forms smooth, more or less cushion-like masses. In these masses, which may be over a centimeter thick, the center is composed of seemingly empty hyphal cells and all development is confined to the peripheral region. Thus in a cross section through one of these masses the surface layer gives much the appearance of a hymenium. The interhyphal spaces are filled with watery ooze which envelops the hyphae to their tips. In stained sections its extent is clearly visible because of granules at the surface. All reproductive struc-

tures develop near the surface within this saturated region. Figure 1 is a somewhat diagrammatic representation of these characteristics as seen in a single section of material abundantly developing spore sacs. In younger material the surface would be occupied by conidial structures.

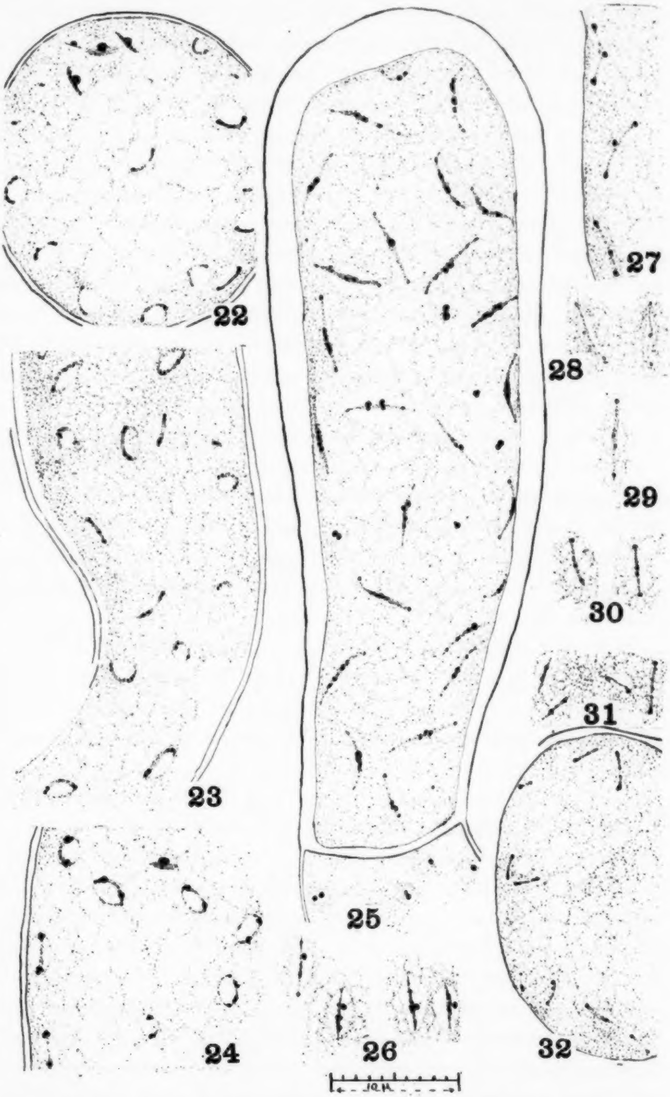
DEVELOPMENT OF SPORE SACS

Typically, spore sacs develop on hyphae which for several cells back are broader at their apical than their basal ends (FIG. 1, many cases, and 7) but many exceptions to this were found (FIG. 1, spore sac at extreme left). In all cases the ultimate cell itself becomes apically enlarged as the earliest definite indication of its differentiation (FIG. 4 AND 5). In some cases at least, the differentiation is evident even before the cross wall below is developed (FIG. 5). The nuclei in all tips, whether about to give rise to spore sacs, conidia, or vegetative growth, are so similar that they are indistinguishable (FIG. 3, 5, 9, AND 10). As a spore sac becomes larger and older an enormous multiplication of nuclei occurs, the walls thicken, and the centrally vacuolate condition is replaced by denser protoplasm (FIG. 4–7, 10, AND 12–15). The nuclei in older spore sacs look like those in older vegetative cells but are surrounded by dense rather than scanty cytoplasm.

Spore sacs vary so greatly in size and nuclear content that it is very difficult to estimate the age of a given spore sac during its earlier stages of development. The thickness of the wall as correlated to nuclear size seemed the best criterion available but it was not entirely dependable. In older spore sacs the wall is much thicker toward the apex than at the base. A series of cross sections beginning with figure 11, a cross section of a hyphal tip, and followed by figures 12–15, show successive changes in the nuclear size and wall thickness up to the beginning of spore formation. So far as could be determined no multiplication of nuclei occurs after the stage shown in figure 15. Along with the multiplication of nuclei in the spore sacs there is very definite evidence of the degeneration of nuclei. In some spore sacs, developed under seemingly optimum conditions, few or no degenerating nuclei are found, while in others, where apparently growth is not so vigorous, they abound. In general, degenerating nuclei show granular,

deeply staining, chromatic masses instead of definite rounded nucleoli. They are considered degenerate because they are found constantly in cells whose cytoplasm shows the coarse granular condition characteristic of dead or dying cells.

Wherever nuclear divisions, which will be discussed later, were observed in spore sacs, all nuclei were dividing simultaneously (FIG. 22–32). Because of this an attempt was made to determine how many nuclear divisions occurred in a developing spore sac. The great variations in nuclear number in spore sacs made the results unsatisfactory. In counting on any one slide and averaging the number of nuclei in broader hyphal tips, young spore sacs with large nuclei, and spore sacs with spores, the numbers indicated three divisions in each case, as 20 for hyphal tips, 40 for young spore sacs, and 160 for mature spore sacs. This would indicate the existence of a stage with 80 nuclei between the 40 and 160-nucleate stages. Accurate counts on spore sacs with small nuclei were never possible because of their minute size and the presence of other chromatic materials in the cell for the counts made would make the estimated number (80) in the series mentioned seem possible. Whether hyphal tips such as these are ever transformed into spore sacs is questionable though possible. At least in many cases apical cells about to form spore sacs are clearly differentiated before the cross wall below is formed. These counts would indicate a series of 20, 40 (80), and 160 nuclei in a sporangium. Thus 3 or 2 divisions occur owing to whether counts on hyphal tips were or were not included. In order to secure more accurate counts on the number of spores in spore sacs than could be secured from serial sections, in the spring of 1933, isolated living spore sacs were mounted in thin films of water and crushed by pressure on the cover glass until the spores, held together by the intersporal slime, were separated so that they were only one deep in the mass. In making such counts from the same materials on two successive days the average number of spores one day was twice as great as for the other. (Thus if materials had been fixed the first day, and counts made, the conclusions would have been entirely erroneous. If counts could have been continued some ratio might have been established but a single hot day killed all materials both outside and in the laboratory.) The spores from



FIGS. 22–32. Mitotic figures commonly observed, 22–24 before thickening of the wall of the spore sac, and 25–32 after thickening of the wall.

twenty-two living spore sacs were counted. The fewest number of spores found in this material was 42 and the largest 160. Counts of similar, large, well developed spore sacs in serial section gave as many as 159 spores. If the nuclei for a spore sac with 42 spores had arisen by 3 successive, simultaneous divisions the tip would originally have had 6 nuclei (6–12 (1 degenerate), 22 (1 degenerate), 42), and the one containing 160 spores would have developed from a tip with 20 nuclei (20–40–80–160) with no degenerate nuclei. Curiously this was the identical range that had been secured in counts of 36 typical hyphal tips from various materials. However hyphal tips do occur with fewer than 6 nuclei and more than 20. As reported in an earlier paper (**14**) one spore sac containing 8 spores was observed. If 3 divisions had occurred this must have developed from a tip containing a single nucleus! On the other hand, many especially broad hyphal tips, such as ordinarily give rise to spore sacs and were arbitrarily listed as young spore sacs, contain about 40 nuclei which may be the initial number. If so there could have been only two simultaneous divisions during the development of a spore sac. No definite cytological evidence was secured by which the point could be settled.

Even if it is impossible to say definitely whether two or three simultaneous divisions provide the nuclei for the spores developed in spore sacs, nevertheless counts show very positively that there is no multiplication of nuclei after the beginning of spore formation.

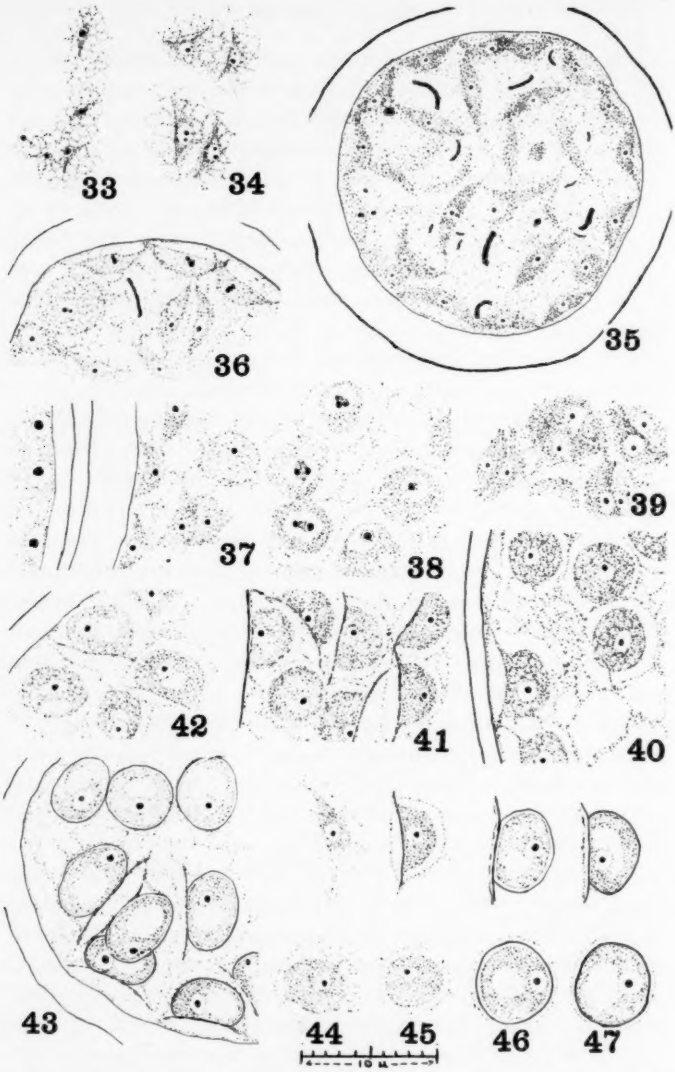
As a result of divisions in the spore sac the nuclei just preceding spore formation are the smallest observed—so small that it is almost impossible to observe details of behavior.

Figure 33 represents a possible beginning of spore formation and figure 34 shows a very definite, early stage in spore formation. The nucleus, which at all times shows indications of not being symmetrical in its organization, becomes surrounded by cytoplasm in a very unsymmetrical fashion. As a result of this, definite lens-shaped protoplasmic masses are formed which lie in thinner cytoplasm. The lens-shape mentioned is that of a circular lens, flattened or concave on one side and convex on the other. Thus when seen in surface view they are rounded and in side view more

or less crescent or spindle shaped. The flattened surface usually lies longitudinally in the sporangium and spores near the wall commonly, but not always, lie with the concave side toward the wall (FIG. 35–37). A clear area, probably a vacuole, is constantly located next to the concave side of the developing spore. The nucleus, during the early stages of spore formation, usually shows a nucleolus and a very small, deeply staining granule. The deeply staining granule may be entirely wanting. When present it may be located on any side of the nucleus as related to the shape of the spore. It has not been possible to attach any significance to its presence. As previously mentioned, many conspicuous, deeply staining, fibrous to crystalline, curved rod-shaped bodies of various sizes often abound in the thin cytoplasm between the spores (FIG. 35 AND 36) but are not universally present. The wall of the spore sac appears thicker at this time than at any other period during development.

The nuclei in these lens-shaped masses of protoplasm gradually increase in size and their surrounding cytoplasm becomes denser and thicker toward the center of the mass (FIG. 37–39). (In figure 37 a part of an adjacent young spore sac is shown for comparison of size of nuclei.) Figure 39 shows a few spores from near the center of a spore sac where they often lie closely appressed, flat surface to flat surface, or variously arranged side by side, seemingly as a matter of chance. In spore sacs that show other indications of degeneration as well as in vegetative cells and old spores the nuclei very commonly take on the appearance shown in figure 38.

In stages shown up to figure 40 the cytoplasm of the young spores is very homogeneous in structure. As the spores become more spheroidal many tiny vacuoles appear (FIG. 40) and each spore mass becomes surrounded on its convex surface by a transparent gelatinous-appearing layer. Soon the wall begins to form around the spore. It appears to form first on the concave side, but this is probably due to the fact that when looking at a side view one looks through the edge of a flattened surface because in surface view the wall there is no more obvious than on other parts of the spore. Curiously the wall over the flattened surface is deposited on the spore side of the clear area (vacuole) developed on



FIGS. 33–47. Stages in the development of endogenous spores. 33–37, 39–43, successive stages in the development of the spores; 38, young spores with degenerating nuclei; 44–47, a diagrammatic representation of spores as seen in side view (above) and surface view (below) in 4 selected stages of development.

the flat side of the spore, but over the rounded surface of the spore the wall begins to be deposited on the outside of the clear gelatinous-appearing area surrounding it (FIG. 41 AND 42). This clear area becomes narrower and narrower until the wall becomes closely adherent to the cytoplasm within the spore (FIG. 43). While the wall is being formed around the spore the vacuoles fuse to form the one large vacuole characteristic of the mature spores. Figures 44–47 show conventionalized spores during four selected stages of development. Side views are shown above and surface views below.

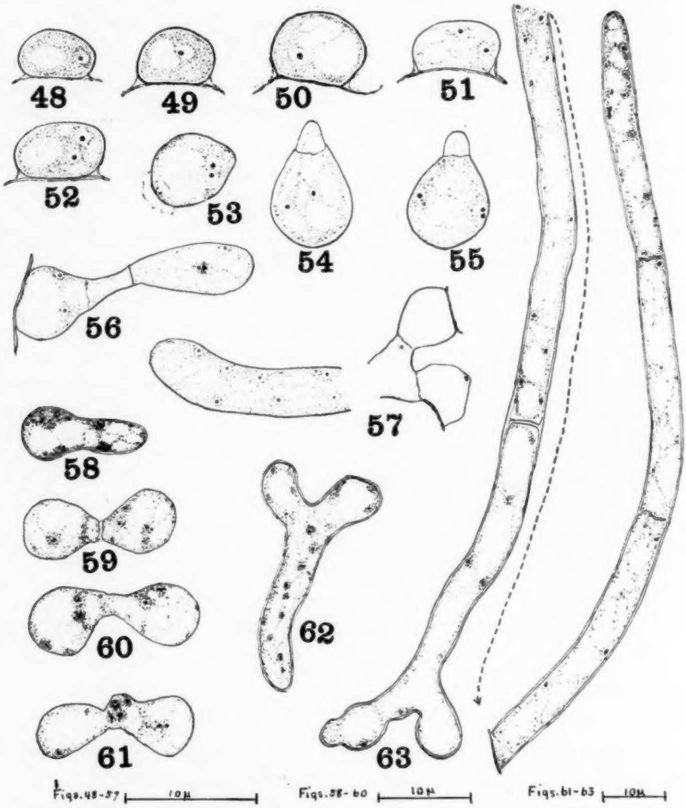
It seems evident that the " hat " shape of the spores results from the curious manner in which the spore walls are formed. The wall over the flattened surface extends to the outer area of the gelatinous appearing layer formed over the rounded surface. As the gelatinous layer becomes contracted as the spore matures the two walls at the edge of the flattened surface form the " brim " of the " hat." The development of the walls of the spores was best seen when stained with haematoxylin and counter stained with orange G in clove oil. The walls of spores stain like other mature walls but are much more impermeable as is shown by the fact that no stain would penetrate the spores when Barrett's smear technique was used.

The oily intersporal slime so conspicuous in fresh materials is soluble in the reagents used in the paraffin method so does not appear. Between the spores and lining the wall of the spore sac is a very delicate granular network that appears to be cytoplasmic (FIG. 35–43). Seemingly the slime is secreted in the meshes of this intersporal cytoplasm. This network becomes less and less definite as the spore sac matures and by the time of discharge is commonly entirely indistinguishable. At no time was a condition in any way resembling cleavage observed in a spore sac.

GERMINATION OF SPORES

In addition to toto mounts of germinating spores a few germinations were observed in sectioned materials. Figures 48–57 show successive stages as seen in sectioned materials, while figures 58–63 are from toto mounts. As was previously reported (**14**) germ tubes may arise from single spores or from the fusion of two

spores. Figure 48 shows a typical mature spore. As germination begins the spore swells, enlarging its vacuole (FIG. 49 AND 50) and the nucleus divides (FIG. 51 AND 52). Figures 53 and 54 show spores germinating while still binucleate, while figure 55



FIGS. 48–63. Germination of spores. 48, a mature spore; 49–57, successive stages as seen in paraffin sections, and 58–63, as seen in toto mounts.

shows a similar germination stage with four nuclei. Figure 56 shows a spore with a germ tube with a single septum and its nuclear content. (The narrow isthmus in the germ tube is due to curvature and part being cut away.) It was almost impossible to

follow older germ tubes in sections. In figure 57, taken from two sections, the only definite case of a germ tube arising from the fusion of two spores, observed in serial sections, is shown. Figures 58–63, illustrating toto mounts, show the same behavior. Grossly these germination stages closely resemble similar stages in yeasts as shown by Guilliermond (**4**), but the nuclear behavior differs widely in that the spore nucleus in *Ascoidea* in all cases observed by the writer divides before germination and gives rise directly to coenocytic cells.[4]

CONIDIA AND THEIR GERMINATION

As has been previously stated conidia develop on hyphal tips which taper apically. From the tip of a hypha a slight enlargement develops which becomes filled with cytoplasm (FIG. 64). As this enlargement increases in size nuclei pass into it from the hypha below (FIG. 65 AND 66). As the conidium assumes its mature form a cross wall is formed to separate it from its bearer. The cross wall at first appears as a clear gelatinous layer at the base of the conidium in the midst of which a granular region of differentiation occurs (FIG. 67). Thus a new wall is formed on each side and the conidium separates readily from the cell below (FIG. 69). As has been described in an earlier paper, the conidia vary greatly in form. Thin-walled, cylindrical conidia such as shown in figure 70, are characteristic of young, vigorously growing hyphae and fall off very readily. Thicker walled, more globular conidia (FIG. 69 AND 71) are common on older hyphae, while especially thick walled conidia, such as shown in figure 72, are apt to form, as here shown, on proliferating tips following sporangial development and on or in other old hyphae. The nuclear content is similar in all cases. Each conidium contains many nuclei situated largely in the peripheral cytoplasm but the center is vacuolate.

Conidial germinations were observed only in toto mounts which were always unsatisfactory. Germinating conidia give rise to coenocytic germ tubes which may continue typical vegetative growth, give rise directly to conidia (FIG. 74), or, after the formation of a few cells, form small but typical spore sacs (FIG. 73 AND

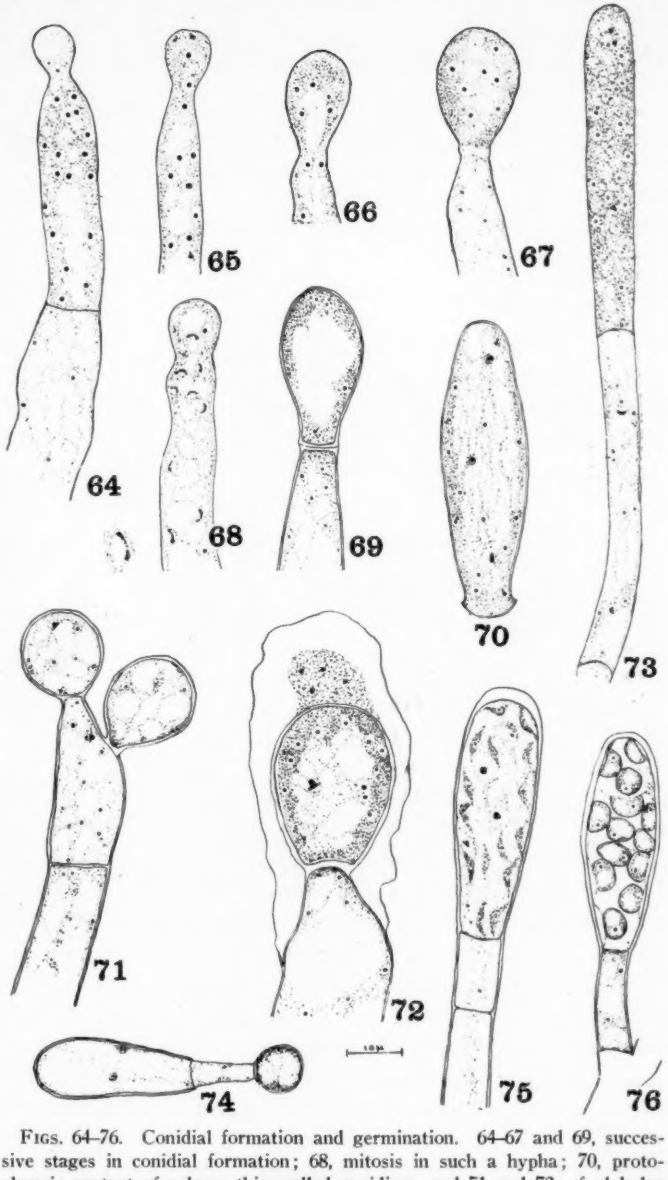
[4] Varitchak (**13**) figures some early germination stages that are uninucleate.

75). Similar small spore sacs may develop from hyphal fragments (FIG. 76).

NUCLEAR DIVISIONS

Feeling that nuclear behavior, especially during the development of the spore sac, might not only be of interest but also helpful in determining the relationships of *Ascoidea,* more time was devoted to a study of this phase than any other. The results have been most disappointing. Chromatic conditions that at least resemble mitoses are commonly observed. In sporangia, where they are most commonly seen, the entire nongranular parts of the cell retain the chromatic stains so tenaciously that it is difficult to get a clear differentiation. Because of this and the fact that the stages observed do not show uniformity in chromatic behavior, the possibility that they are degenerative stages has been given much consideration. It seems probable, however, that they are mitotic stages because normal nuclei are never found in spore sacs showing such conditions. The granular cytoplasm in such cells seems perfectly normal. These stages have been considered mitotic by other workers on *Ascoidea* and will be discussed in this light.

Similar mitotic stages have been observed in spore sacs and occasionally in vegetative cells. In spore sacs the divisions appear to be simultaneous while in vegetative cells this is not usually true. In figures 22–32 an attempt has been made to show as accurately as possible the types of stages most commonly seen in spore sacs and the extent of the chromatic variations commonly seen in the same spore sac. These probably represent stages in the last mitosis preceding spore formation. Figures 22–24 show what appears to be a curious type of intranuclear spindle. In some cases (FIG. 22) the spindle seems rather short with granules at the poles while in others the spindle forms a nearly complete circle within the nucleus with very tiny granules opposite the heavier part of the spindle (FIG. 24, above and to right). Stages such as these were occasionally observed in vegetative cells (FIG. 68). When seen in spore sacs they always occur before the walls are greatly thickened. If the stages shown in figure 24 are properly interpreted, it seems probable that with the rupture of the nuclear membrane and the straightening of the spindle, stages such as are shown in figure 25 might result.



FIGS. 64–76. Conidial formation and germination. 64–67 and 69, successive stages in conidial formation; 68, mitosis in such a hypha; 70, protoplasmic content of a long, thin-walled conidium, and 71 and 72, of globular thicker-walled conidia still attached to the cells which produced them; 74, a small conidium being formed at once from a germinating conidium; 73 and 75, small spore sac formed at the tips of short hyphae following germination; 76, a similar small spore sac formed on a short outgrowth from a hyphal fragment.

Stages similar to those in figure 25 are more commonly seen than any other stages. The spindle is always more or less curved. That these spindles are later in development than those shown in figure 24 is evident by the greatly thickened wall. It seems possible that the rupture of the nuclear membranes might release pressure upon the inner layer of the wall of the spore sac, which is gelatinous, and thus permit its sudden expansion. Four nuclei from a similar spore sac are shown in figure 26. Here it appears definitely as if the nucleolus was being ejected. Stages, somewhat similar to these, occurring in a hyphal tip, are shown in figure 16. Figures 27–33 show stages that undoubtedly develop later than those shown in figures 25 and 26.

Varitchak (**13**) as a result of his studies gives the sequence in nuclear division shown in figure 77, copied photographically from

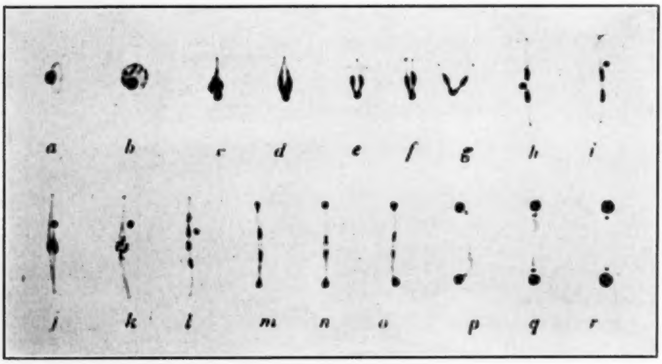


FIG. 77. Mitotic stages. A photographic reproduction of Varitchak's Fig. 2.

his figure 2. The nuclei shown in his figure 2, " a " and " b," are undoubtedly what the writer holds to be degenerating nuclei. For his figures " c–g," stages such as shown in figures 22–24 are possible. The writer would suggest stages such as are shown in figure 78 as a more probable series. Stages similar to all of Varitchak's (**13**) have been observed but those similar to his earlier figures appear to be degenerative stages. From his " h " on, his figures are characteristic except that when the chromosomes have reached the poles two definite chromatic bodies are often observed.

Nuclei such as shown in figures 8, above, 15, 19–21, 34, etc., have been a serious problem. It would seem that they might represent young nuclei that have just divided, such as might be expected to follow figures 30–32, and it may be that this is the case, as seen in figures 33–36. Very commonly, however, nuclei at this

78

FIG. 78. Mitotic stages. A series of stages suggested as a result of these studies.

stage show only the one definite chromatic granule. Nuclei of this type are seen most commonly in old and depleted cells where the nuclei are small and active growth is not taking place. Their occurrence would indicate that they more probably represent either degenerative conditions or a type of amitosis in which figures 19–21 would constitute a series.

DISCUSSION

Forms such as *Ascoidea* that do not obviously fall within any of the usually recognized major groups of fungi are always of especial interest to students of fungous phylogeny. In this case the interest has centered around the spore sac and its endogenously developed spores. Three types of spore sacs are known among fungi: sporangia, asci, and gametangia. The spore sacs of *Ascoidea* were considered sporangia by Lohwag (**8**) who holds that *Ascoidea* is a true Phycomycete while Varitchak (**13**) concludes that *Ascoidea* is a primitive Ascomycete closely related to *Dipodascus albidus*. That the spore sac of *Ascoidea* was the result of the development of one gametangium in the absence of the other has been suggested by Atkinson (**1**).

From the description just given it is obvious that *Ascoidea* is not a typical Phycomycete. Its spore sacs resemble sporangia in that they are multinucleate from the beginning, develop without

nuclear fusions and at maturity contain a variable number of spores. The spore sacs of *Ascoidea* differ widely from sporangia in that the spores are not formed by progressive cleavage and at maturity are surrounded by cytoplasm in whose meshes an oily slime is secreted. While grossly this condition in the mature spore sac resembles the condition in certain Mucors where the intersporal spaces are filled by an oily slime, development is very different. Luhwag's studies (**8**) of *Ascoidea* did not include cytological observations on spore formation and this doubtless accounts for his conclusion that the spore sac is a sporangium and that *Ascoidea* is a true Phycomycete.

The spore sacs of *Ascoidea* are also surely very different from typical asci. No nuclear fusions were observed but simultaneous divisions occur in the spore sac. No astral rays were observed during spore delimitation but the spores are formed by a curious sort of free-cell formation and at maturity lie embedded in cytoplasm, similar to that surrounding ascospores in typical asci. Varitchak (**13**) has reported the fusion of two specialized nuclei in the young spore sac of *Ascoidea* and that this fusion nucleus by repeated divisions gives rise to the nuclei of the spores while all other nuclei degenerated. Hundreds of hyphal tips in all stages of development up to spore formation have been critically examined but no fusions of nuclei were ever observed. If the spores were formed by the repeated divisions of a fusion nucleus up to twenty (or forty) nuclei would regularly degenerate during the development of the larger spore sacs. Careful counts have shown that this was not the case. In old materials where developmental conditions were poor so large a percentage of degenerating nuclei might occur but in vigorously growing materials spore sacs abounded in which no indication of any degenerating nuclei were found. Thus indirectly, for the materials used in these studies, we have evidence against the probability of fusions and degenerations, as described by Varitchak (**13**).

The suggestion of Atkinson (**1**) that the spore sac of *Ascoidea* might represent an apogamously developed gametangium remains to be considered. In *Saprolegnia ferax* and related forms the oögonium regularly develops apogamous oöspores without the presence of antheridia. In such forms oöspores may even develop

in sacs looking like sporangia. In all cases in this genus the oöspheres arise by cleavage and there is no cytoplasm surrounding them nor the so-called oöspores which develop from them. In closely related forms the oösphere is surrounded by an abundant periplasm formed seemingly by the cytoplasm and degenerating supernumerary nuclei. In looking over the wide variations known to occur among Phycomycetes it would be easy to conceive of coenogametangia similar to the young spore sacs of *Ascoidea* in which many, instead of one, oösphere was differentiated and in which the oöspheres were surrounded by cytoplasm. The fact that two simultaneous mitotic divisions seem to precede spore formation in *Ascoidea* and in oögonia is at least an interesting coincident.

It has been commonly suggested by those who would derive the Ascomycetes from the Phycomycetes that the ascus may have arisen by the modification of the gametangium. In this connection, *Dipodascus* with its many-spored ascus arising from the fusion of a single nucleus from each coenocytic gametangium, has been taken as the simplest known form. Into a series such as this, *Ascoidea* as here described can easily be considered a related form in which the spore sac is a gametangium developed apogamously. The fact that no nuclear fusion was observed during the development of the spore sac does not in the mind of the author in any way detract from this relationship. The literature dealing with the subject has been so well discussed by Varitchak (**13**) that it will not be reviewed here.

It is surely such fungi as *Ascoidea* that form the links in a chain connecting Phycomycetes with Ascomycetes. Its phycomycetous relationships are obvious but it is even more definitely related to the lower Ascomycetes. Its two most outstanding ascomycetous characteristics are (1) its hyphae in which cross walls are not only present but formed so near the tip of the hyphae that the apical cells are usually, at the time of formation, the shortest cells in the hyphae, and (2) its spores are formed by free-cell formation. Harper's statement (**6**) that free-cell formation is perhaps "the most important and specific feature by which to distinguish the ascus from other spore producing cells" is in general held by students of fungi. There are, however, no universally accepted criteria for the determination of border line forms such as *Ascoidea*.

Schröter (**10**) divided the Ascomycetes into three primary subdivisions. Hemiascomycetes, Protoascomycetes, and Euascomycetes. Into the first two groups he placed all forms lacking ascocarps. In a more recent classification Gäumann and Dodge (**3**) have in general included in the one group, Hemiascomycetes, the forms divided by Schröter into Hemi- and Protoascomycetes. In doing this the Hemiascomycetes are distinguished from Euascomycetes primarily by the presence of ascogenous hyphae in the latter group and their absence in the former. Two orders of Hemiascomycetes are recognized: the Endomycetales, which include those forms " in which the ascus arises directly as a product of the sexual act (wherever this takes place)," and the Taphrinales. It seems to the writer that *Ascoidea* definitely belongs in the Endomycetales as characterized above. Gäumann and Dodge (**3**) recognize three families in the Endomycetales, the Dipodascaceae, the Endomycetaceae, and the Saccharomycetaceae. The close relationship of *Ascoidea* to *Dipodascus albidus* of the Dipodascaceae has been previously pointed out. In the genera of the Endomycetaceae the hyphae are typically uninucleate, except when very young, but *Endomyces Magnusii* has coenocytic cells and even conidia. *Ascoidea's* budding type of conidial formation and germination show characteristics common in species of the Endomycetaceae and Saccharomycetaceae. In these groups also there is a marked tendency towards apogamy, and in some known forms such as *Endomyces javanensis* and *E. capsularis* no nuclear fusions are known. If, in some strains, nuclei fuse in the young sporangium, as reported by Varitchak (**12 and 13**) this would only place it more definitely in line with the known variations in this order.

Another fact that connects *Ascoidea* definitely with the Endomycetales is the curious, hat-shaped spores which closely resemble those of *Eremascus fertilis, Endomyces decepiens, E. fibuliger, E. Lindneri,* and *Willia anomala.* Not only do the mature spores resemble each other but Guilliermond (**5**) and Mangenot (**9**) also figure and describe young unwalled spore masses of similar form and a similar type of development. It seems probable that more detailed studies will show even greater similarities. One point of difference is that Guilliermond (**5**) always shows the flattened surface of the spore in some other position than toward the wall, as is

the most characteristic position for spores near the wall in the spore sacs of *Ascoidea*. Even the nuclei of these forms seem similar in all details so far as given. It is interesting to note that while free cell formation is described for all related forms, astral rays have not been observed. While of no especial significance, the fact that many of the well known members of the Endomycetales occur in slime fluxes or sugary solutions is an interesting coincident.

The confusion that has existed concerning *Ascoidea* seems only natural because it is neither a typical Ascomycete nor a typical Phycomycete. It seems to the writer, as also to Varitchak (**13**), that *Ascoidea* is closely related to both *Dipodascus* and *Endomyces* of the Endomycetales. The writer differs from Varitchak (**13**) in finding no fusions in the spore sac. Because no fusions were found, the writer considers the spore sac an apogamously developed ascus of a type intermediate between the asci of *Dipodascus* and *Endomyces*. The coenocytic characteristics of *Ascoidea* suggest a close relationship to *Dipodascus* of the Dipodascaceae, but spore formation is so like that in *Endomyces* that *Ascoidea* might as readily be placed in the Endomycetaceae as tentatively placed by Gäumann and Dodge (**3**). Because the ascus of all members of the Hemiascomycetes is so different from typical asci it seems fitting that, as suggested by Varitchak (**13**), and used by Gavaudan and Varitchak in recent publications, we should distinguish such asci by calling them *hemiasci* and the spores *hemiascospores*.

SUMMARY

1. For these studies materials collected at Ithaca, New York, in August, 1927, and at Lincoln, Nebraska, in June, 1930–1933, inclusive, were used.

2. The hyphae, conidia, and spore sacs are coenocytic but the spores formed in the spore sac are uninucleate.

3. The nuclei are much larger in young cells than older cells but all are similar in structure and are characterized by a large nucleolus and little additional chromatic material.

4. Characteristic chromatic bodies of several types are conspicuously present in coenocytic cells. Some of these at least arise from degenerated nuclei. The most conspicuous of these is a

granular disk to which a homogeneous highly refractive globule is centrally attached. These are evidently the bodies considered specialized nuclei by Varitchak.

5. Active growth is apical; new cells are formed as soon as the apical cell has elongated so as to be about a third longer than a typical cell. Cells below the apical region have scanty protoplasm and older cells lose their protoplasmic content.

6. All reproductive structures on actively growing thalli are apical, the surface of the thallus often appearing like a vague hymenium.

7. Hyphal tips contain up to 20 or more nuclei, young spore sacs up to 40 nuclei, and mature spore sacs up to 160 spores.

8. The nuclei for the spores formed in spore sacs arise by simultaneous divisions.

9. Spores are first differentiated in spore sacs as lens-shaped masses of denser protoplasm, flattened on one surface and rounded on the other, and are surrounded by less dense cytoplasm.

10. The characteristic hat-shape of the endogenously developed spores is due to the fact that the wall of the spore is formed next to the dense cytoplasm of the spore initial on its flat surface and on the outside of a clear layer of gelatinous appearance over the rounded surface. As the gelatinous layer disappears the " brim " of the " hat " is formed where these walls unite.

11. The spores formed in spore sacs enlarge and become two to several nucleate before forming germ tubes. They give rise to coenocytic hyphae either directly or after fusions.

12. Conidia of all types are multinucleate. They give rise either to conidia by budding or to coenocytic hyphae.

13. Nuclear divisions in the sporangium are probably mitotic, and probable stages are discussed. Similar figures were occasionally observed in vegetative cells. There are also some indications of amitosis.

14. The spore sac is probably an apogamously developed ascus.

15. These studies indicate that *Ascoidea* is a true Hemiascaceous fungus closely related to *Dipodascus* and *Endomyces*. The term hemiascus can well be applied to such asci.

The writer wishes to extend thanks to Prof. H. M. Fitzpatrick, of Cornell University, for his continued interest, and for sugges-

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THE ASCOCARPS IN SPECIES OF PENICILLIUM

C. W. Emmons

(with 16 text figures)

Some form genera of the Ascomycetes have such definite conidial characters that their members appear to be of common origin. *Penicillium* may be placed in this category. Despite differences in type of conidial apparatus the species of *Penicillium* have many points in common so that this genus, considered wholly on the basis of the conidial stages of its species, properly circumscribed, has been believed to rest upon a somewhat different basis than many others. Over 400 species of *Penicillium* have been described on the basis of the conidial stages. The ascocarpic structures in the genus are relatively little known. The abundance of species of *Penicillium* and their economic importance justify further studies of this group. In view of these facts it is proposed to examine more critically some of the species which produce ascocarps. Such a study reveals certain extremely interesting features that appear during the development of the ascocarpic stage. Differences in the ascogonia and asci can be correlated with the group differences pointed out by Thom (**1930**); but these differences indicate lines of development more clearly than do the differences in conidiophores, as would be expected. Dr. Charles Thom has furnished several of the strains studied, and the author is also indebted to him and to Dr. B. O. Dodge who suggested this comparative study for helpful suggestions given during this investigation and the preparation of the manuscript.

Taxonomic studies of the *Penicillia* have necessarily been based for the most part on the imperfect stage. Few species complete their full life cycles in culture, and it will probably continue to be desirable as heretofore to prepare keys largely based upon the conidial apparatus so that those engaged in cultural and biochemical studies can recognize and identify the forms encountered. A few ascocarpic species have been described, but many of these

descriptions lack certain details which are essential to a better understanding of the group as a whole. Seventeen strains and twelve species of *Penicillium* and one species of *Byssochlamys* are included in this study.

During the early course of this study two lines of development seemed apparent. In one series of forms the perithecial wall was formed of interlacing hyphae which in some species were closely knit, but in others were loose or so scanty as to be hardly apparent. The ascocarp continued to expand, presumably by intercalary growth of the hyphae composing the wall so that the mature ascocarp might be many times the size it had attained when the first asci were formed. This series was further characterized by an end to end arrangement of the asci to form chains. The ascocarpic initials varied and the ascospores were marked in various fashions, although most were spiny.

The second series bore ascocarps which were sclerotium-like, the asci appearing in a cavity which formed at the center. The ascocarp in these forms reached a definitive size before the first asci appeared at its center and the thick-walled pseudoparenchymatous cells which made up the perithecial wall appeared to be incapable of further cell division. The asci in this series were borne on short stalks as side branches from the ascogenous hyphae. Occasional asci appeared end to end, but this was exceptional (**Dodge 1933**). In this series the ascocarp was initiated in a uniform manner at the crotch of a tree-like system of hyphae.

So sharply and completely were these two series differentiated that a generic separation was at first deemed advisable. However, the study of other species subsequently received from Dr. Thom indicates that the gap between the two can be bridged and has yielded further important information about this group of Ascomycetes. The information now at hand concerning the origin and development of the ascocarp in these forms gives a basis for a much better understanding of the genus *Penicillium*. It is still inadequate, however, for a revision of the genus or the creation of new generic names for the ascocarpic species. All species included in this study, even though they seem to follow divergent lines, are therefore referred to *Penicillium* unless they have been re-

ferred by their authors to some other genus. Likewise no revision of species is made, except that one form is described as new.

The salient features of the forms included in this study are shown in the diagram of text figure 16. It will be seen at once that most of the species under consideration fall into one of two groups, one group characterized by an ascocarp not at all sclerotized and enclosing asci borne in chains, the second by an ascocarp made up of pseudoparenchymatous tissue which, following the digestive activities of the ascogenous hyphae, subsequently contains stalked asci within a central cavity. In the first two species the structure which appears to function as an ascogonium is merely a slightly differentiated portion of the vegetative mycelium and is probably a structure simplified through reduction. In the next four species the ascogonium is highly differentiated. In the next six species a simple substitute type of ascogonium reappears but is borne upon a specialized system of branched hyphae, present also in the other species but in less well developed form.

The disposition of asci in chains would seem to be a character of considerable theoretical importance. Although it may have arisen as a reduced form from the more common type, it is strikingly different and a comparative examination of the asci alone of *Penicillium Wortmanni* and *P. Brefeldianum*, for example, would suggest that they belong not only in different species, but even in different families. On the basis of this and correlated differences the species first examined in this study were, as stated above, arranged in two distinct groups with nothing in common between them so far as the ascocarp was concerned. Later when *P. egyptiacum* was received and examined it was found that the nature of the ascocarp would place it in one group, while its asci resembled those of the other group. These twelve species can therefore be arranged, not in two unrelated groups, but along two divergent lines. *Byssochlamys fulva* does not seem to belong in either series.

However, the divergence that can occur within a genus is a problem requiring further study, and it can be solved only by a careful morphological study of the structures involved in reproduction. Until a large number of species have been thus studied it is unsafe to attempt revision of the genus *Penicillium*. This

study has tended to confirm Thom's group separation based on penicillial characters, but has shown some wide variations in reproductive structures within the biverticillate group. The final evaluation of these differences awaits examination of other forms. There is a greater range of variations among the Plectascales than within some other groups of Ascomycetes, and some of these differences appear to be important. For example, in *Thielavia terricola* there appears to be a crozier and nuclear fusion in the ascus, while in *T. sepedonium* neither is present (**Emmons 1932**). The loss of certain structures and the acquisition of new ones may occur with greater frequency than has been supposed.

Besides the variety of structures appearing within the genus certain variations are to be noted in some species. One of the most significant of these is the tendency toward loss of ascospore production. Within a few weeks after *Carpenteles asperum* had been received in this laboratory it had ceased to produce ascocarps in some subcultures. It was only from certain tubes, and from fertile sectors on cornmeal agar plate cultures that a fertile strain was recovered. *Penicillium vermiculatum* exhibited a similar tendency. In one of the two original strains fertility has in some measure been restored. The second now produces only conidia. Non-ascosporic strains have arisen repeatedly also in *P. Brefeldianum. P. egyptiacum* has also given a form predominantly conidial. These species are homothallic, as proved by many single spore isolations. Presumably, therefore, sterility is not caused by segregation of two complementary strains. This sheds some light on the problem of sterility (so far as ascospore production is concerned) in the many strictly conidial species of *Penicillium* in nature. Contrary to the data of Derx, the author has found not the slightest evidence of heterothallism in any of the species of *Penicillium* examined. *Penicillium avellaneum* is not included in this study because the available strain had become sterile. Probably, therefore, the great majority of species of *Penicillium* are non-ascosporic, not because they are haplonts, but because at the time some variation or mutation gave rise to the strain, or at a later time, there was a concomitant loss of fertility. Without attempting any explanation of the mechanism of this loss, we are yet justified in assuming that it occurs for such loss of fertility can

be observed in variants arising in the laboratory. It is reasonable to assume that the same forces operate in nature to produce strictly conidial strains of *Penicillium*.

The chains of asci merit some general description. Their arrangement in *Penicillium egyptiacum* seems to differ in no essential respect from that in the other forms, and since somewhat clearer preparations have been obtained of this species it will be used as the basis for a general description. Crushed aceto-carmine mounts have been the most useful method for study. In such mounts many of the groups of asci are either too compact and complex for an understanding of their relationships, or they are too much broken up. If properly made, however, aceto-carmine preparations show some short chains of asci so clearly that there can be no doubt of their arrangement. These chains consist usually of not more than 5 or 6 cells and of these, two or three will show ascospores in some stage of development (FIG. 9E).

The chains are spirally coiled, or at least curved, and in the younger cells the wall toward the center of the spiral is the more convex. In some cases the inner tip of the coil can not be clearly seen, in some cases it appears to be broken off, and in still others it may be slightly turned back to form a hook (FIG. 9D). In spite of its superficial resemblance to the organ which initiates ascus formation in many Ascomycetes, it is not a crozier, and careful search of the young asci has failed to reveal any vestige of such a structure. The asci are definitely end to end, and such an arrangement does not follow the intervention of a crozier of the conventional type. End to end arrangement might follow crozier formation if the ascus developed from the antepenultimate instead of the penultimate cell, but it does not seem necessary to postulate any such complicated sequence here. The ascus seems, rather, to develop directly from a cell of the ascogenous hyphae. It may be that some substitute for the crozier mechanism occurs earlier in the development. In the young ascogenous hyphae certain protuberances and hook-like structures bear a superficial resemblance to croziers, but it does not appear that they function in the manner of croziers.

The nuclear story for these *Penicillia* has not been elucidated, but all are homothallic. From 5 to 20 single spores, ascospores

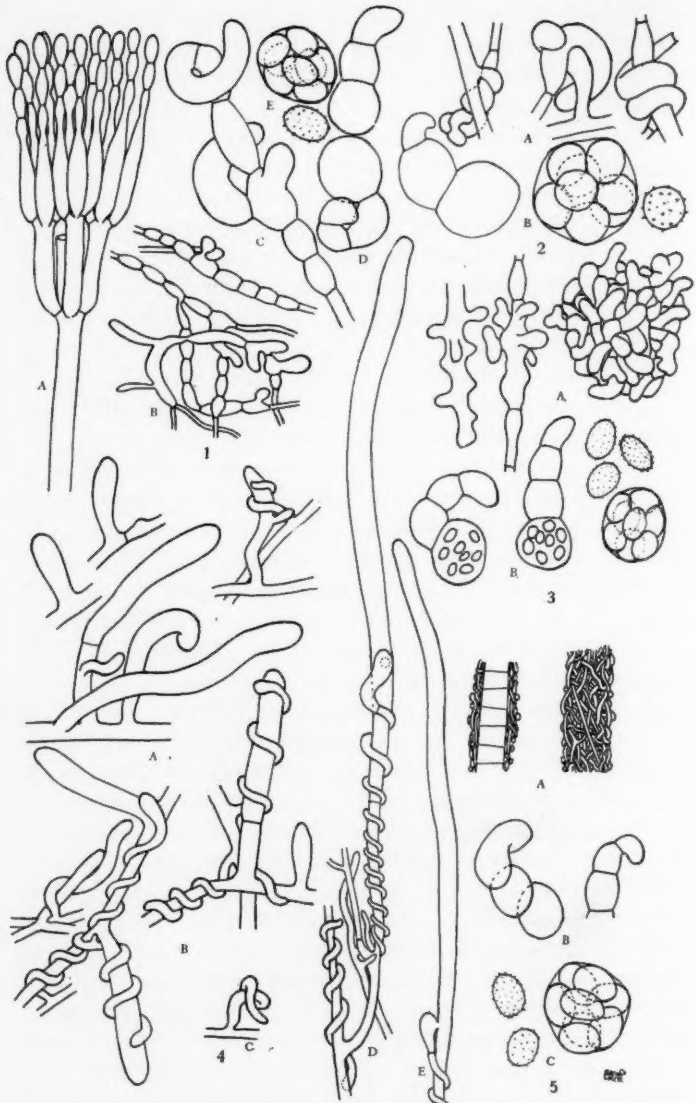
and conidia, have been isolated from each strain and in all species ascospores have been produced in such single spore cultures. As noted previously ascocarpic strains frequently lose the power to produce asci in culture.

Each of these 18 strains, with the exception of two, which are referred to a new species herein described, is typical of the species to which it is assigned, and seven are subcultures from the original strains described in papers cited. It will not be necessary, therefore, to give complete details of each one. It will be sufficient to consider each in turn and to point out some features, perhaps for the first time observed, which seem to be of phylogentic significance.

Penicillium Wortmanni Klöcker 1903 (FIG. 1).

The ascocarp of *Penicillium Wortmanni* is very simple, like that of a *Gymnoascus*. There is no real perithecial wall. The ascogonium from which the ascocarp arises is also a simple structure, probably a reduced form. Its appearance is preceded by an increased vegetative development so that on cornmeal agar plates the ascogonia are to be found in hyphal tangles, which can be readily seen when one looks down upon the plate with the low power of the microscope. This hyphal development is particularly striking in forms in the second series considered here, and will be described in connection with those species.

Within these growths of aerial hyphae intercalary segments or side branches of the mycelium become swollen and cut up into short cells. In this condition they take up the stain more deeply (FIG. 1B, C). In most Ascomycetes, some change, conditioned by environment, age, and other factors, which we are accustomed to think of as maturity, is followed by the appearance of specialized structures, the ascogonia, and usually, antheridia. In *P. Wortmanni* a cell or slightly differentiated branch of the vegetative mycelium functions as an ascogonium, and no paired organs are to be found. This condition of maturity is not confined even to a single branch or to the progeny of its cells arising by cell division. It appears to spread to neighboring cells and to nearby hyphae. The growth of ascogenous hyphae is thereby initiated at many points within a region which in its later development assumes the character of a single ascocarp.



FIGS. 1–5. *Penicillium.*

The differentiated cells may be terminal or intercalary cells (FIG. 1B). Any given ascocarp may arise from one such plexus or from the fusion by centripetal growth of two or more such fertile regions. On cornmeal agar it is often possible to make out individual ascocarps with walls which are very loose in structure, but definitely specialized. On richer media the fertile regions are often quite extensive and limited only by the incrusted hyphae which surround them, without definite boundaries. The development of the perithecial wall is either very limited or lacking. Such a loose weft of hyphae as is present is probably supplied by neighboring hyphae without any close connection with the ascogenous hyphae. The ascocarp is limited in its growth only by nutritional conditions. Paraffin sections as well as teased mounts show that asci may develop while the fertile region is quite small, and that this fertile region or ascocarp increases in size by peripheral growth long after the first asci appear.

The asci are typically in chains (FIG. 1D). In exceptional cases asci appear in this species and in others of this type, as side buds or terminal cells on a branch, but they are not ordinarily on stalks even in these cases. The terminal cell of a chain of young asci at certain stages of development is often turned back upon itself (FIG. 1D). This suggests a crozier, although the exact relationship is very difficult to make out. This arrangement of asci and the possibility of a crozier mechanism has been discussed above. The eight ascospores are spiny (FIG. 1E). This species was proved by the writer to be homothallic.

Penicillium spiculisporum Lehman 1920 (FIG. 3).

The strain of *Penicillium spiculisporum* coming from Porto Rico, upon which this study was based, has already been described (**Kesten, et al. 1932**). It seems to be typical of the species. The structure preceding the formation of the ascocarp is similar to that seen in *P. Wortmanni* (FIG. 3A), but is somewhat more specialized, and it arises in the midst of a branching hyphal system which is more highly developed in *P. egyptiacum* and will be described in connection with that species. Near the center of this loose vegetative hyphal system a side branch or an intercalary portion of a hypha swells, shows characteristic staining reactions, and branches profusely. These branches are short, gnarled, and form

a compact mass (FIG. 3A). From this hyphal knot develops an ascocarp varying greatly in size and possessing a well differentiated perithecial wall of interlacing hyphae of several cell layers in thickness. This definite perithecial wall does not, however, wholly restrict further growth of the ascocarp. The latter may increase in size during the formation of asci. Presumably this growth is permitted by intercalary growth of the compact weft of hyphae making up the wall. The eight-spored asci in this form also are formed end to end. The ascospores are spinulose (FIG. 3B). The species is homothallic.

Penicillium bacillosporum Swift 1932 (FIG. 2).

The ascocarp of *P. bacillosporum* arises from a pair of short coiled hyphae (FIG. 2A). These are comparable to the primordia in *P. stipitatum* described below; but unlike that species the ascocarp develops directly around the primordium. The asci, like others in this series, lie end to end to form short, curved or coiled chains. The eight spores are globose, and the walls are marked by comparatively coarse spines (FIG. 2B). The mature ascocarp of this species has been fully described by Swift (**1932**). The homothallic nature of this species was proved by the describer and was verified in the present study.

Penicillium vermiculatum Dangeard 1907 (FIG. 4 AND 5).

Two strains of this species have been studied. One was isolated from normal skin in a survey to determine the prevalence of yeast-like fungi in normal individuals (**Benham and Hopkins, 1933**). The second was received from Dr. Westerdijk under the label *Arachniotus ruber*. The rediscovery of the species at this time is of interest as confirmation of Dangeard's description, and its peculiar morphologic features make it a particularly interesting form in connection with the present study.

The ascogonium and antheridium of *P. vermiculatum* form a striking picture. Since Dangeard described it there have been few references to it. Derx (**1925**) identified a culture received from Thom with this species, but without giving any description of it. If examined at the time the ascogonia are forming it could not be mistaken for any of the other species included in this study. The ascogonium is a long clavate cell reaching an occasional length of 250 μ (FIG. 4A, B, D, E). The antheridium which Dangeard

calls the "trophogone" arises usually from a separate, more delicate hypha, and coils around the base of the ascogonium several times (FIG. 4B, D, E). An enlarged terminal cell is cut off and this comes into open communication with the ascogonium through a large pore (FIG. 4D, E). The various stages in this development and some of the less common branched forms which are found are shown in these figures.

Dangeard described and figured the early stages in ascocarp formation in this species in great detail. The primordia which he described are radically different from those described by Zukal, Brefeld and others who have studied *Penicillia* and from all other forms studied here. The very definite organ which he found fusing with the ascogonium was assigned to the rather indefinite function of serving as a "trophogone." While Dangeard admitted that this cell fusion occurred between the two organs he denied that there was any fertilization by a nuclear fusion. It is very gratifying to be able to confirm in many details Dangeard's report on the origin and development of the ascocarp in this species, although we are compelled to believe that there is an actual copulation, leading to sexual reproduction; or, in other words, Dangeard's "trophogone" is, in fact, a true antheridium.

The ascogonium becomes septate and enveloped in closely wound delicate hyphae (FIG. 5A). According to Dangeard's report most of the isodiametric cells in the upper three-fourths of the ascogonium give rise to ascogenous hyphae. The young ascocarp is consequently at first club-shaped and then elliptical. Dangeard states that mature ascocarps are elliptical. In our cultures on cornmeal agar plates the ascocarps finally become spherical and reach a diameter of 1 mm. The ascocarp has a definite wall several cell layers in thickness. The asci are borne in chains and are eight-spored (FIG. 5B, C). Dangeard figures asci with not more than six spores, but states in the text that the asci are eight-spored. After being in culture for several months the first of these strains became less fertile and produced smaller ascocarps. In cultures from single ascospores the fertility has been in some measure restored. This species as well as the others studied, is homothallic; single ascospores readily produce ascospores again. The ascospores are spiny (FIG. 5C).

Penicillium stipitatum Thom sp. nov. (FIG. 6 AND 7).

Mycelio homothallico; perithecio globoso, 130–400 μ diametro; ascis ovoideis, 5.5–6.5 $\times$ 7–8 μ, 8-sporis; ascosporis ellipticis, 2–2.2 $\times$ 3–3.6 μ, marginato.

Colonies on Czapek's solution agar floccose tufted in yellow (luteus) shades passing over to orange or even red orange shades in age; reverse yellow to red orange; aerial hyphae studded with granules yellow in the young and growing period becoming reddish in age; conidial apparatus irregularly biverticillate with sterigmata up to 10 by 2 μ taper pointed and closely packed in the verticil; conidia about 3.5 μ in long axis, rather thick walled fusiform smooth; ascogenous masses enveloped by tufts and masses of yellow hyphae, within which hyphae are arranged into a fairly definite wall forming a brittle and easily crushed perithecium; asci 8-spored, ripening quickly, 5.5–6.5 $\times$ 7–8 μ.

Ascospores 3–3.6 μ in long axis by about 2 μ, lens-shaped consisting of a two valved body with an equatorial band or frill about 0.5 μ in width, usually appearing single but occasionally apparently double with a groove partially evident between them.

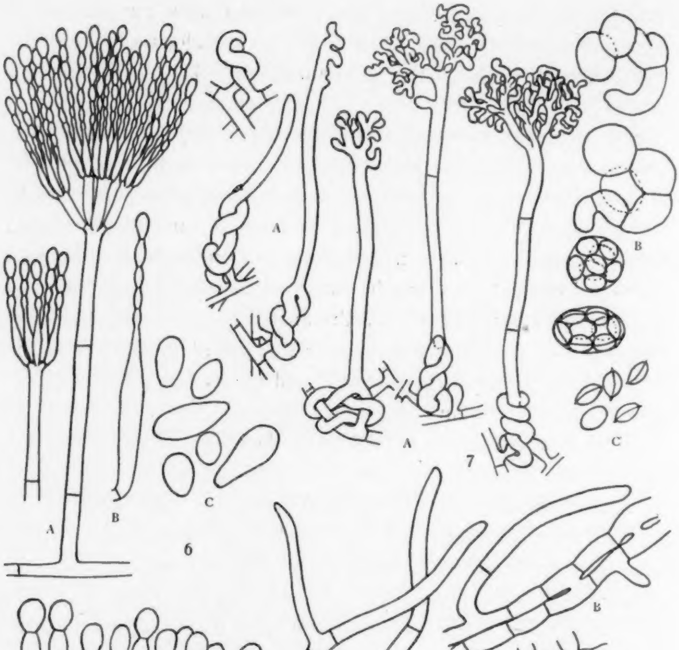
Cultures have been deposited with the American Type Culture Collection and with the Centraalbureau voor Schimmelcultures, Baarn.

Two strains of this fungus have been studied. One was isolated from rotting wood in Louisiana and was sent to Dr. Thom's laboratory by T. C. Sheffer. The second was sent to him by Dr. Ma from Peiping, China. Because of distinctive features which could not be identified with any other *Penicillium* this form is described as a new species.

The two strains of this fungus which have been studied vary slightly in growth habit and in some measurements. They are too nearly alike, however, for specific differentiation. The second produces larger ascocarps and more conidia. Conidia in both strains are formed sparingly. The elliptical conidia are borne on biverticillate penicilli (FIG. 6A). The sterigmata are long and tapering, as in others of this group (FIG. 6B). The ascocarpic initial consists of a pair of short hyphae which complete one or two coils about each other and fuse by a large permanent pore. The resulting hypha elongates to about 100 μ and the ascocarp

forms at its tip (FIG. 7A). The perithecial wall is composed of interlacing hyphae which do not become pseudoparenchymatous. The 8-spored asci are borne in chains (FIG. 7B). Each ascocarp is encircled longitudinally by a flange (FIG. 7C).

The ascocarpic primordium of *Penicillium stipitatum* is a most remarkable structure, and, so far as known, is without parallel in other forms. In *Aspergillus herbariorum*, according to Kny and DeBary, the mature ascocarp is raised upon a single hyphal thread support, but here the ascocarp originates from primordia developed at the top of this hyphal stipe instead of at the base as in *Penicillium stipitatum*. Furthermore, in *Aspergillus* the stalk supports the mature ascocarp, while in *Penicillium stipitatum* the cells of the stipe become vacuolate, and the ascocarp is supported, rather, by surrounding vegetative hyphae. In such forms as *Sclerotinia* where the ascogonial coil is developed in the sclerotium, the ascocarp is supported on a stipe, which is, however, composed of many hyphae, some of which, at least, are gametophytic. In *Penicillium stipitatum* two similar or barely differentiated hyphae arise as side branches, usually from different hyphae, and coil around each other (FIG. 7A). After about two turns they fuse by a large pore so that the opening is of a diameter equal to that of the inside of the hypha. This opening is permanent. The two branches that initiate this development are without doubt the ascogonium and the antheridium. From our knowledge of what takes place in other fungi we may assume that fertilization takes place at this point. Cytological proof in this case has not yet been obtained. We would now expect the development of an ascocarp around this structure as in other *Penicillia*. The actual development is quite different. One of the branches, or the hypha arising from the union of the copulating branches, elongates until it reaches a length of 100–150 μ (FIG. 7A). It becomes once or twice septate, and at its tip begins to put out branches. These gnarled branches are formed in profusion, become septate, and by their further branching and intertwining, form a more or less compact mass not unlike the ascocarpic initial of *P. spiculisporum*. Within this ascocarp ascogenous hyphae appear and give rise to asci arranged end to end in chains (FIG. 7B).



FIGS. 6–9. *Penicillium.*

The ascospore of *Penicillium stipitatum* is also remarkable. It is surrounded by a flange which is very delicate and extends some distance beyond the spore wall (FIG. 7C). In a few exceptional cases two parallel rings appear to be present. The spores lie in the ascus in such a manner that the flange is parallel to the ascus wall. Cytological preparations, while not entirely satisfactory, do show, at an earlier stage, the eight nuclei of the ascus distributed through the cell and arranged so that their beaks radiate toward the ascus wall. The flange may be an extension of the kinoplasm, and at one stage possibly forms a continuous spherical membrane inside of and parallel to the ascus wall. The flange is not a surface configuration which arises after the spore is otherwise fully matured, but in stained sections shows early in spore development.

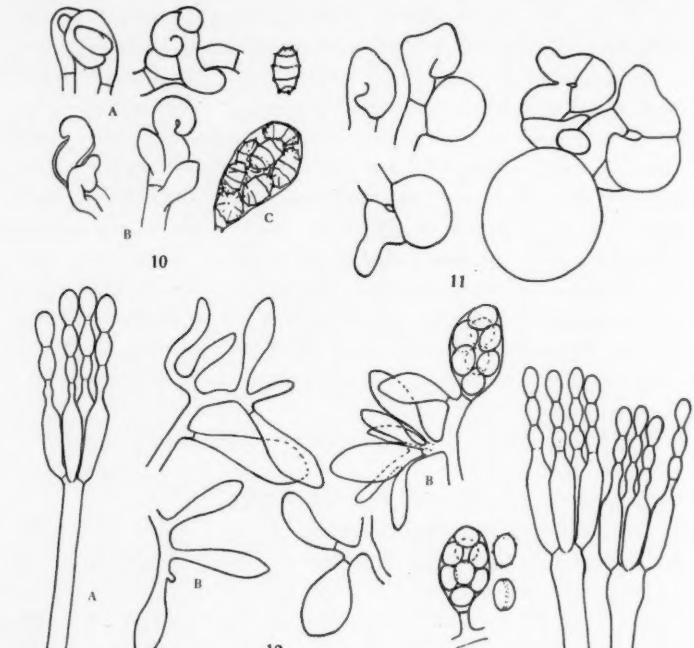
Penicillium stipitatum is homothallic as proved by numerous single spore isolations.

Penicillium luteum Zukal 1889 (FIG. 10).

This strain was sent from Manitoba by Dr. Bisby to Dr. Thom. It is apparently the species described by Zukal since it conforms to the description he gave.

The ascocarpic initial is composed of a pair of short coiled hyphae (FIG. 10A). These complete one or two turns and the ascocarp develops directly around them. More than one pair of initials may become involved in the formation of one ascocarp. The perithecial wall is composed of a very loose weft of hyphae. The ascocarp continues to increase by peripheral growth and by the accretion of adjacent young ascocarps after the first asci appear. The fertile regions of a colony, which are bright yellow, as in *P. Wortmanni* are covered by specialized encrusted hyphae. In *P. luteum* these hyphae are wavy and sometimes spirally coiled.

In spite of its superficial resemblance to *P. Wortmanni* an examination of the young asci of *P. luteum* reveals a very different organization. While the asci of the former species are borne end to end, those of the latter are borne as sessile side buds from an ascogenous hypha. Their appearance is therefore very different also from that in the *P. Brefeldianum* series since the conspicuous stalks are absent. The arrangement of the ascogenous hyphae and the young asci is to some extent obscured by a brown pig-



FIGS. 10–15. *Penicillium.*

mentation in these structures and by a layer of gelatinous material associated with the walls. It appears, however, that croziers are present (FIG. 10B) and that asci arise through them.

The development and nature of the ascus suggests that *P. luteum* is not closely related to the other forms studied here. The extreme reduction in its conidial apparatus also suggests this view. Many of the fractional penicilli are reduced to single cells bearing a chain of spores. It may be, however, that when other forms are found and studied some species will be found to bridge the gap here as was the case in the two other types of ascus arrangement which seemed at first fundamental. Derx (**1925**) states that this species is heterothallic. Our single spore cultures have produced abundant ascospores. This evidence, and the fact that several other homothallic species of *Penicillium* may become non-ascosporic in culture, indicate that Derx probably encountered, not heterothallism, but some type of variation.

Penicillium egyptiacum van Beyma 1933 (FIG. 8 AND 9).

Two strains of this species have been available, presumably from the same original source. One was sent to Dr. Thom from Dr. Westerdijk; the second was transmitted to us by Dr. Thom as van Beyma's type. Because it combines some of the characters of the two types of *Penicillium* outlined above, and because it corresponds in so many respects with the fungus described by Brefeld as *P. glaucum* this is probably the most important species included in this study.

The penicillus of *Penicillium egyptiacum* is monoverticillate or sometimes asymmetrically biverticillate, and it bears the short, abruptly tapering sterigmata which characterize this series of forms (FIG. 8A).

The ascocarpic initial of *Penicillium egyptiacum* is like that described by Dodge for *P. Brefeldianum*. The details of its structure can be made out more easily here than in some other species. When this fungus is grown on cornmeal agar plates at 30° very few conidia are produced, and there is a good production of ascocarps. When an appropriate zone near the edge of a colony grown under such conditions is examined under the low power of the microscope the only aerial hyphae to be seen may be these ascocarpic initials. They appear as upright branching

systems arising from large hyphae in the substrate (FIG. 8B). If some of this material is mounted in aceto-carmine the branches making up this system take up the stain more deeply than the other hyphae. They are long, slender and somewhat antler-like (FIG. 9A). Transverse septa are at first far apart. At a later stage these branches, and particularly the inner ones, stain more deeply with aceto-carmine, numerous cross septa appear, many branches develop, and hyphal anastomoses are frequent (FIG. 9B, C). The hyphal anastomoses may be found in other parts of the mycelium, but they are more numerous in this region and it seems probable that they are of some significance in ascus production. The ascocarp now rises directly from the center of this hyphal plexus. There are no paired organs, but the repeated branching and intertwining of these hyphae, now cut up into short cells, form a compact mass of pseudoparenchymatous cells. Young ascogenous hyphae resembling those figured by Brefeld, appear in a few days at the center of the ascocarp (FIG. 9D). The peculiar type of branching these assume is distinctive. Cell fusions can sometimes be found, but these are not constant features. Despite the crooked contorted nature of these hyphae and the chain of asci into which the cells develop, the sequence can sometimes be made out (FIG. 9E). The asci are certainly arranged end to end, although they are often somewhat coiled. The ascospores, eight to an ascus (contrary to van Beyma's account), are surrounded by a longitudinal furrow bordered by flanges (FIG. 9F). Upon germination they swell enormously and split along the furrow (FIG. 9G).

In its type of ascocarp, in the character of its ascogenous hyphae, in the arrangement of asci in chains, and in type of ascospore *Penicillium egyptiacum* is like *P. glaucum* of Brefeld. Our strains, however, do not have the type of ascogonium Brefeld figured, they produce asci promptly, and they do not produce typical apple rot. The ascospore markings are perhaps less conspicuous. *P. egyptiacum* is more nearly like Brefeld's fungus than is *Carpenteles asperum* which has recently been put forward as that species. In any case we now know that Brefeld must have been correct in describing a *Penicillium* with a sclerotium-like ascocarp enclosing asci disposed in chains, and ascospores with

longitudinal furrows splitting on germination like those of *Aspergillus.*

Single spore isolations have proved that this species is homothallic.

Penicillium Ehrlichii Klebahn 1930 (FIG. 14).

This strain was subcultured from Klebahn's type. Its ascogonium and ascocarp are of the same type as those of *P. egyptiacum,* but its asci are borne as side branches from the ascogenous hyphae (FIG. 14A). It is, then, a representative form in the second series of ascus-bearing *Penicillia* except that the ascocarp in *P. Brefeldianum* and *Carpenteles asperum* become much harder. The ascocarpic initial of *Penicillium Ehrlichii* is like that of *P. egyptiacum* but the hyphal branches are somewhat longer and more delicate. The differentiation of the hyphae at the center of this hyphal system appears to follow the same order in the two species. The ascospore of *P. Ehrlichii* shows a longitudinal furrow bordered by prominences and the entire surface is marked by spines (FIG. 14C).

The homothallic nature of *Penicillium Ehrlichii* was proved by numerous single spore isolations.

Penicillium Brefeldianum Dodge 1933 (FIG. 13).

The ascogonium of *Penicillium Brefeldianum,* as Dodge pointed out, arises in the crotch of a tree-like growth of hyphae. The exact morphological structures at this point are very difficult to make out. The innermost branches of this system seem to swell and to take on characteristic staining properties as in the less differentiated hyphae of *P. Wortmanni.* In some, and perhaps all, of the forms of this type there are hyphal anastomoses in this pleux (see *P. egyptiacum*). From some of these structures, which may function as primary ascogenous hyphae so well described by Brefeld, the ascogenous hyphae arise; from others the sterile tissue which surrounds the ascogenous hyphae. The eight-spored asci are borne on stalks (FIG. 13A, B). The species is homothallic. The further development of this form has been fully described by Dodge (**1933**).

Penicillium javanicum van Beyma 1929 (FIG. 12).

The development of two strains studied is as given by van Beyma (**1929**) and Dodge (**1933**). One of these is van Beyma's

original strain and the other was sent to Dr. Thom by Dr. Ma from Peiping, China. Both are 8-spored but correspond otherwise with van Beyma's description. In all respects save measurements, color, and degree of sclerotization this species is much like *P. Brefeldianum.* The asci form on stalks (FIG. 12B). Under the proper conditions of lighting and magnification a thin region can be made out extending around many of the spores in a manner to suggest a valve (FIG. 12C). This band is not, however, bordered by wings. Both strains are homothallic.

Carpenteles asperum Shear 1934 (FIG. 15).

This strain has been recently described by Shear (**1934**). He considered this to be the form Brefeld studied (**1874**) and the title of his article reads, "*Penicillium glaucum* of Brefeld (*Carpenteles* of Langeron) refound." However, because of uncertainties regarding Link's type he proposed a new name, *Carpenteles asperum* adopting the generic name proposed by Langeron (**1922**). Shear reconciles the incomplete agreement between this form and that described by Brefeld by the statement, commonly accepted, that Brefeld was working with impure cultures. Whether Brefeld's cultures were pure or impure may never be known, but there is now good evidence at hand for believing that his story of the development of "*Penicillium glaucum*" is correct, save perhaps in the matter of the primordium and conidia. This evidence is supplied by an examination of *P. egyptiacum.* While *P. egyptiacum* is not actually the species described by Brefeld, it at least coincides so closely with it that the facts reported here amount to a virtual verification of Brefeld's data.

The ascogonium of *Carpenteles asperum* arises as in the preceding forms in the crotch of a system of hyphal branches, and the early development is similar to that of *P. Brefeldianum.* The sclerotium-like ascocarp becomes so hard, however, at certain stages, that it is very difficult to crush it under a cover slip, and good paraffin sections are almost impossible to secure. Asci do not usually appear for several weeks. The ascospores present the double wing described by Brefeld (FIG. 15B). The asci are however, formed on stalks (FIG. 15A) rather than in chains. Single spore isolations prove the species to be homothallic.

Penicillium Gladioli Machacek 1927.

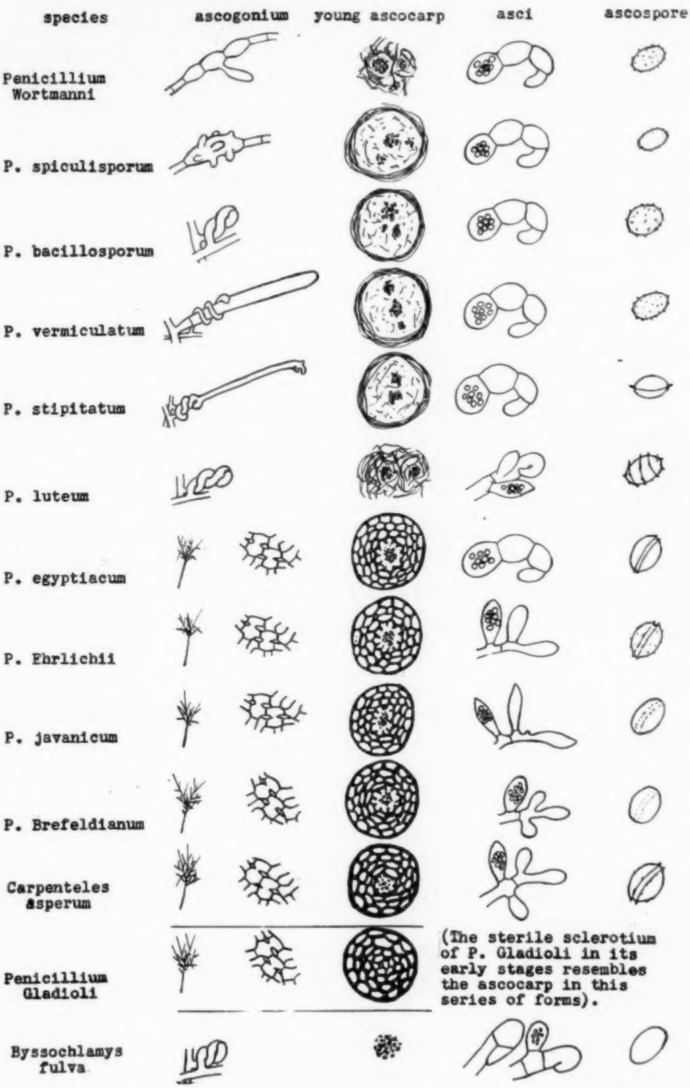


FIG. 16. Diagrammatic representation of the ascocarpic structures in *Penicillium*.

A strain of *Penicillium Gladioli* is included for comparison although no asci have been found in our cultures. The abundant sclerotia remain sterile. This strain was isolated by the authors from a *Gladiolus* corm. The primordium of the sclerotium arises in the crotch of a tree-like system such as Dodge described for *P. Brefeldianum*. The subsequent development also is like that species except that asci do not appear at the center of the sclerotium. The sclerotia of *P. Thomii* and similar forms have not been studied.

Byssochlamys fulva Olliver and Smith 1933 (FIG. 11).

Byssochlamys fulva differs widely from other forms studied here in conidial as well as ascocarpic structures. The conidiophore is that of the *Paecilomyces varioti* series. The ascocarp lacks a perithecial wall and the asci are borne in clusters, forming tiny white flecks, easily seen among the brown conidia. These asci are preceded by the formation of a conspicuous crozier (FIG. 11) so that the young ascogenous hyphae of this species are very different in appearance from those of the other forms studied here. *Byssochlamys fulva* does not fit into either of the series outlined here.

The fungus is homothallic but there is a tendency toward sterility in strains propagated by either mass or single spore transfers.

SUMMARY

A morphologic study of the reproductive structures involved in the ascomycetous phase of 12 species (17 strains) of *Penicillium* and of one related species has revealed a surprising diversity in ascogonia and antheridia, in types of ascocarps, in the disposition of the asci, and in ascospore markings. Two divergent lines appear, but there are species having some of the characters of each line. The ascocarpic initials of *Penicillium Wortmanni* and *P. spiculisporum* are barely differentiated segments of the vegetative hyphae. Those of *P. bacillosporum* and *P. luteum* are paired coiled organs. In *P. stipitatum,* described as a new species, the fusion of the ascogonium and antheridium results in the formation of a hypha 100–150 μ long at the tip of which the ascocarp forms. The ascogonium of *P. vermiculatum* is a clavate cell, reaching a length of 250 μ, around the base of which the antheridium coils. In *P. egyptiacum, P. Ehrlichii, P. javanicum, P. Brefeldianum,*

and *Carpenteles asperum,* the young ascocarp arises from the innermost branches of a tree-like system of branches. *Penicillium Gladioli* represents the sterile sclerotium forming species. *Byssochlamys fulva* has a short hyphal coil from which the asogenous hyphae arise, and through the intervention of croziers naked asci develop. In the first seven species the asci are in chains and in the next four they are on stalks. All species studied are homothallic.

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EXPLANATION OF FIGURES

Fig. 1, *Penicillium Wortmanni:* A, Conidiophore. × 1300; B, Ascogonia. × 500; C, Ascogonium. × 1300; D, Chains of young asci. × 1300.

Fig. 2, *Penicillium bacillosporum:* A, Ascogonia and antheridia; B, Asci in chains, a mature ascus, and ascospores. × 1300.

Fig. 3, *Penicillium spiculisporum:* A, Ascogonia and young ascocarps; B, Asci in chains, a mature ascus, and ascospores. × 1300.

Fig. 4, *Penicillium vermiculatum:* A, Young ascogonia. × 1300; B, Young ascogonia and antheridia. × 1300; C, Young antheridium. × 1300; D, Mature ascogonium and antheridium. × 500; E, Mature ascogonium and antheridium in profile. × 500.

Fig. 5, *Penicillium vermiculatum:* A, Portion of a very young ascocarp in optical section and surface view; B, Chains of young asci; C, Mature ascus and ascospores. × 1300.

Fig. 6, *Penicillium stipitatum:* A, A monoverticillate and a biverticillate conidiophore. × 500; B, A sterigmata. × 1300; C, Conidia. × 1300.

Fig. 7, *Penicillium stipitatum:* A, Stages in the development of the ascogonia. × 500; B, Chains of young asci. × 1300; C, Mature asci and ascospores, the spores shown in both views. × 1300.

Fig. 8, *Penicillium egyptiacum:* A, Conidiophore. × 1300; B, Habit sketch of the vegetative development preceding appearance of the ascocarp. × 100; C, Mature conidia.

Fig. 9, *Penicillium egyptiacum:* A, Hyphae in the primordium of the ascocarp; B, Hyphal fusions in the ascocarp primordium; C, A later stage in the development of the primordium; D, Ascogenous hyphae; E, Chains of asci; F, Ascospores, face and side views; G, Germinated ascospore. × 1300.

Fig. 10, *Penicillium luteum:* A, Ascogonia and antheridia; B, Ascogenous hyphae; C, Mature ascus and ascospore. × 1300.

Fig. 11, *Byssochlamys fulva,* ascogenous hyphae and young asci. × 1500.

Fig. 12, *Penicillium javanicum:* A, Conidiophore; B, Young stipitate asci; C, Mature ascus and ascospore. × 1300.

Fig. 13, *Penicillium Brefeldianum:* A, Young asci; B, Mature asci and ascospore. × 1300.

Fig. 14, *Penicillium Ehrlichii:* A, Young asci; B, Mature ascus; C, Ascospores. × 1300.

Fig. 15, *Carpenteles asperum:* A, Young asci; B, Mature ascus and ascospore; C, Conidiophore. × 1300.

FIG. 16, Diagrammatic representation of the ascocarpic structures in *Penicillium.*

THE GENUS DICHEIRINIA [1]

George B. Cummins

(with plate 16 and 1 text figure)

The genus *Dicheirinia* was established in 1907 by Arthur (**1**) with *Triphragmium binatum* Berk. & Curt. as the type and only species known. Both uredia and telia were known at that time but the available material was too scanty to allow a detailed study. In 1925, when part 10 of volume 7 of the North American Flora was published, Arthur reported numerous other collections but considered to bear only uredia and telia.

Jackson (**5**) in 1931 added a second species, *D. superba* Jacks. & Holw., and made a substantial contribution to the knowledge of the genus by showing that subcuticular pycnia consistently occurred with the microtelia. The present study adds two species by transfer from other genera, confirms Jackson's discovery of pycnia, shows *D. binata* to be macrocyclic with uredinoid aecia and offers an emended description of the genus to accord with these facts.

PYCNIA AND AECIA

The pycnia are subcuticular, as pointed out by Jackson (**5**), and occur rather abundantly among the aecia in *D. binata* and among the microtelia in *D. superba* and *D. manaosensis*. Development of the following spore form often obliterates all trace of the preceding pycnia and doubtless accounts for the tardy recognition of the kind of life cycle.

Uredinoid aecia are known only in *D. binata* but are of common occurrence. In the Arthur Herbarium the finest development of aecia is shown on *Erythrina Crista-galli* L., collected July 7, 1924 at the Agricultural Experiment Station, Mayaguez, Porto Rico by Whetzel, Kern and Toro as no. *2415*.

Discal paraphyses like those in the uredia are present in the aecia but are less abundant and may be scarce. The aecia are

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

borne on distorted and hypertrophied veins and petioles or less commonly on the leaf blade. There being no peridium growth is rather indeterminate and may be locally systemic.

UREDIA AND TELIA

Subepidermal uredia are present in *D. binata* and *D. Ormosiae* and offer peculiarities in structure of paraphyses worthy of note. Those of *D. binata* are discal while in *D. Ormosiae* they are peripheral. The paraphyses are unevenly knob- or clove-like in *D. binata* (PLATE 16, FIG. 3) and while specifically distinct easily recall the paraphyses described in other genera. Those of *D. Ormosiae,* however, are unique in so far as the writer is aware. The terminal portion, corresponding to the head in most paraphyses, is profusely branched and dendritic with the ultimate branches tubercle- or finger-like (PLATE 16, FIG. 4). The degree of subdivision varies but may be great. A sorus encircled by these tangled structures appears to be possessed of a peridium (TEXT FIG. 1), and is as sharply limited in extent as though a peridium were present. In young sori the spores are completely covered by the mass of paraphyses but with maturity are liberated through a pore-like opening formed by the upward and outward development of the paraphyses.

Telia are subepidermal with paraphyses as in the uredia. In the macrocyclic species the sori are small and scattered but in the microcyclic species simulate the habit of aecia, occurring on hypertrophied areas among the pycnia and, in *D. superba,* covering extensive areas and apparently arising from a locally systemic mycelium as indicated by Jackson (**5**). While the material is too scanty to allow a definite statement the same development seems likely in *D. manaosensis.*

Sculpturing on the teliospores, while differing in the various species, shows a marked similarity in all (PLATE 16, FIG. 1, 5, 7, 9) being coarsely cubical or digitate and more numerous above. The structure of the pedicel provides the character most distinctive of the genus. The basal stalk is relatively long and fragile, early collapsing to liberate the spores. Shortly below the junction of the pedicel and the spores a horizontal septum is formed dividing the pedicel into a lower and an upper portion. The upper portion

may consist of a single cell (*D. Ormosiae*, PLATE 16, FIG. 5, 6), of two cells (*D. binata*, PLATE 16, FIG. 1, 2; *D. superba*, PLATE 16, FIG. 9, 10) or of three cells (*D. manaosensis*, PLATE 16, FIG. 7, 8) to which the teliospores are directly attached. The pedicel breaks at or below the horizontal septum leaving the teliospores, when mature, carrying only the apical cells of the pedicel. These may collapse to such an extent that no indication of a pedicel remains on mature spores.

Each teliospore is provided with one apical germ pore placed next the vertical walls separating the spores. The sculpturing of the spore wall often makes observation of the pore difficult and in spite of careful study its location in *D. Ormosiae* remains doubtful. This uncertainty offers the only substantial obstacle to the ready acceptance of this species as a member of the genus.

RELATIONSHIPS OF THE GENUS

In naming the genus Arthur (**1**) placed it in the tribe Raveneliatae of the Aecidiaceae and Dietel (**4**) treated it in the same manner, assigning it to the tribe Raveneliae of the family Pucciniaceae. According to Dietel's scheme the genus merits a position between *Diabole* and *Uromycladium* and near *Sphenospora* and *Diorchidium.*

In a study of the genera probably most closely related to *Dicheirinia* one is impressed by the similarity of the teliospores of species of *Diorchidium* and of *Hapalophragmium*. *Diorchidium* differs in having no apical cells on the pedicel and in most species by a lateral rather than an apical placement of the germ pores. *Diorchidium Piptadeniae* has the pores apical and except in possessing a continuous pedicel is similar to *Dicheirinia superba*. *Dicheirinia manaosensis* appears much like *Hapalophragmium setulosum* and *H. ponderosum* in that three cells are borne together. In *Hapalophragmium*, however, the pedicel is continuous and bears a 3-celled teliospore with two cells joined to the pedicel and the third superimposed upon the basal pair. The similarity between *Dicheirinia* and *Hapalophragmium* is perhaps more apparent than real.

In the possession of an apical cell in the teliospore pedicel *Dicheirinia* closely simulates *Diabole*, but differs in other charac-

ters. The latter genus has a pedicel usually bearing several pairs of teliospores, each pair attached to a single apical cell of the pedicel but the spores are more definitely independent of each other and have pores probably lateral. Despite careful study the location of the pores could not be definitely established. It seems probable that there are two pores in each cell, somewhat below the equator, one at the inner angle, the other opposite in the outer wall. If this assumption proves correct the differences between *Dicheirinia* and *Diabole* are considerable. The sori are subepidermal in *Dicheirinia* and subcuticular in *Diabole*. The writer believes the relationship to be more remote than the discussion by Dietel (**3**) would indicate.

The frequent occurrence of a 2-celled condition in the teliospore-heads of *Ravenelia simplex* Diet. offers an opportunity for comparison. The association of the two cells, the distribution of sculpturing and especially the structure of the pedicel, as given by Dietel (**2**, PLATE 6, FIG. 20C), parallel the same characters in *Dicheirinia*. As Dietel (**2**) has pointed out the presence of the several-celled heads gives the only indication of its generic position. The 2-celled condition in *R. simplex* is typical of that found in *Dicheirinia superba*.

While Dietel (**4**) is justified in indicating a close relationship to *Diabole* and *Uromycladium* the writer believes that the situation would be more correctly depicted by considering that *Dicheirinia* in its simpler condition (*D. Ormosiae*) closely approaches such a species as *Diorchidium Piptadeniae*. The insertion of a single apical cell in the teliospore pedicel of *D. Ormosiae* offers the only obstacle to its inclusion in *Diorchidium*. In its more complex species *Dicheirinia* seems definitely related to the genus *Ravenelia* through such a simplified condition as that mentioned above for *R. simplex*. Dietel (**3**) has previously pointed out the correspondence between the apical cells of the pedicel in *Dicheirinia binata* and the cysts in *Ravenelia*.

TAXONOMIC TREATMENT OF THE SPECIES OF DICHEIRINIA

DICHEIRINIA Arthur N. Am. Flora **7**: 147. 1907.

Pycnia subcuticular, conic, hemisphaeric or crust-like, without paraphyses. Aecia when present subepidermal, uredinoid, without

peridium but with few to many paraphyses; aeciospores borne singly on pedicels, the walls colored and echinulate, pores distinct. Uredia when present subepidermal, with paraphyses; urediospores borne singly on pedicels, the walls colored and echinulate, the pores one or more, basal or equatorial, distinct. Telia subepidermal, with few or many paraphyses; teliospores two or three, united laterally on a common pedicel, the walls colored and coarsely tuberculate or digitate, with one apical pore, the pedicels having 1–3 apical cells.

Type species, *Triphragmium binatum* Berk. & Curt., on an undetermined plant (*Erythrina?*).

Arthur's definition of the genus is no longer adequate to care for the species which are here included. The discovery of pycnia and aecia necessitates an expansion of the generic characters. Microcyclic and macrocyclic species must both be considered. The presence of three teliospores in *D. manaosensis,* the consequent three apical cells in the pedicel and the fact that *D. Ormosiae* has only one apical pedicellate cell have all to be provided for. The above emended generic description is presented in light of present knowledge.

The genus is limited to the tropical regions of North and South America.

KEY TO THE SPECIES OF DICHEIRINIA

Teliospores borne two on a pedicel.	
Teliospore-pedicel with one apical cell; macrocyclic.	
Teliospore-pedicel with two apical cells.	1. *D. Ormosiae.*
Teliospore-sculpture digitate; macrocyclic.	2. *D. binata.*
Teliospore-sculpture cubical; microcyclic.	3. *D. superba.*
Teliospores borne three on a pedicel; microcyclic.	4. *D. manaosensis.*

1. **Dicheirinia Ormosiae** (Arth.) comb. nov.

Puccinia Ormosiae Arth. Mycologia **9**: 78. 1917.
Dicaeoma Ormosiae Arth. N. Am. Flora **7**: 391. 1920.

Pycnia and aecia unknown. Uredia hypophyllous, scattered or more or less grouped, at first whitish from the mass of paraphyses, later chestnut-brown, surrounded by a dense peripheral rim of whitish paraphyses, appearing peridium-like (TEXT FIG. 1), paraphyses profusely branched and expanding into a dendritic or botryoid head (PLATE 16, FIG. 4), large, colorless but refractive to light; urediospores irregular, obovoid with the pore in face

view, triangular with pore in lateral view, 20–26 by 24–32 μ; wall dark cinnamon-brown, 1.5 μ thick, somewhat thicker at hilem, sharply and sparsely echinulate, pore one, near the hilem, distinct. Telia hypophyllous, grouped like the uredia but chocolate-brown, paraphyses as in the uredia; teliospores (PLATE 16, FIG. 5, 6) one-celled, borne in closely united pairs (rarely 3) on a common pedicel, oblong or ellipsoid, flattened on the inner side, 15–20 μ wide by 25–32 μ high; wall chocolate-brown and nearly opaque, 3–4 μ thick including the tubercles, coarsely tuberculate with ir-

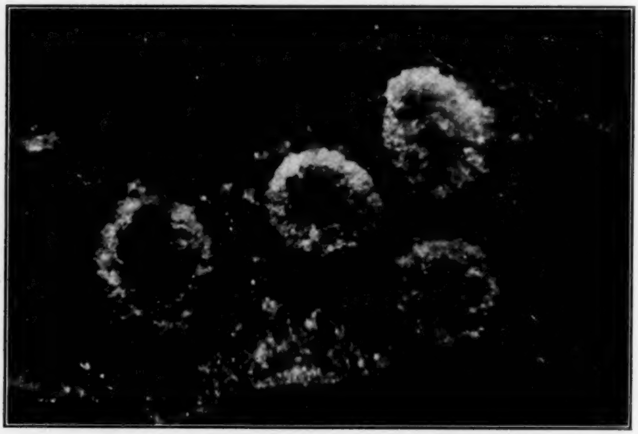


FIG. 1. Telia of *Dicheirinia Ormosiae* showing the dense, encircling, botryoid paraphyses typical of the species. For an illustration of a single paraphysis see plate 16, figure 4. The uredia are identical in gross appearance. × 30.

regular or cubical bead-like warts, the pore one, very obscure but apparently apical and next the inner angle; pedicel bearing one apical cell, rarely two cells by a vertical septum, colorless, fragile, breaking below the apical cell or at the spore.

HOSTS AND DISTRIBUTION: *Ormosia Krugii* Urban, in Porto Rico and Santo Domingo.

TYPE LOCALITY: El Yunque, Porto Rico, on *Ormosia Krugii.*

EXSICCATI: R. Cifferi, Mycoflora Domingensis *34.*

The fragility and early disjunction of the pedicel probably account for the description of this species under the genus *Puccinia.* Details can be studied satisfactorily only in sections when

the proper orientation of the teliospores is obtained. Occasional association of three spores on a pedicel occurs. The apical cell of the pedicel is then usually and perhaps always divided once, the third spore being borne by one cell, the other two by the second cell.

2. DICHEIRINIA BINATA (Berk. & Curt.) Arth. N. Am. Flora **7**: 147. 1907.

Triphragmium binatum Berk. & Curt. Proc. Am. Acad. **4**: 125. 1858.

Lecythea pezizaeformis Berk. & Curt. Proc. Am. Acad. **4**: 127. 1858.

Diorchidium binatum De-Toni, in Sacc. Syll. Fung. **7**: 736. 1888.

Uredo ? pezizaeformis De-Toni, in Sacc. Syll. Fung. **7**: 856. 1888.

Uredo Cabreriana Kern & Kellerm. Jour. Myc. **13**: 25. 1907.

Pycnia amphigenous and petiolicolous, subcuticular, conical becoming flat and wide spread. Aecia subepidermal, amphigenous and petiolicolous, on hypertrophied areas, large, becoming confluent over extended areas, uredinoid, cinnamon-brown, with few to many discal paraphyses; aeciospores borne singly on pedicels, obovoid-globoid or with one side flattened, 19–26 by 29–35 μ; wall 2.5–3 μ thick or slightly thicker above, sharply echinulate, dark cinnamon-brown, the pores 3 with 1 usually on the flattened side and 2 on the curved side, equatorial, distinct. Uredia subepidermal, mainly hypophyllous, light cinnamon-brown, with numerous discal, branched paraphyses having a thick-walled or solid, refractive, irregular knob-like head (PLATE 16, FIG. 3); urediospores like the aeciospores but more globoid, 22–29 by 28–32 μ, with wall thicker, 3–4 μ, and light cinnamon-brown. Telia subepidermal, chestnut-brown, with paraphyses as in the uredia; teliospores (PLATE 16, FIG. 1, 2) in pairs on a common pedicel, obovoid or nearly globoid, flattened on the inner side, 26–29 μ wide by 35–40 μ high; wall chestnut-brown, 2–3 μ thick at sides, thicker above with digitate projections, more abundant above, few or none at the base, the pore one, near the inner angle; pedicel hyaline, with two apical cells, usually breaking below the apical cells.

HOSTS AND DISTRIBUTION: *Erythrina glauca* Willd., in Porto Rico, Cuba, Central America and South America; *E. Crista-*

galli L., in Porto Rico; *E. umbrosa* H. B. K., in British West Indies; ? *E.* sp. in Nicaragua.

TYPE LOCALITY: Nicaragua, on unknown host (? *Erythrina*).

ILLUSTRATIONS: Dietel, Ann. Myc. **24**: 130. 1924, *fig. 1;* Dietel, in E. & P. Nat. Pfl. 2nd Aufl. **6**: 68. 1928, *fig. 52, C.*

An apparently common species usually collected only in the urediosporic stage which is characterized by the shape of the urediospores and the clove-like paraphyses. The teliospores are the largest and most coarsely sculptured of any in the genus.

3. DICHEIRINIA SUPERBA Jacks. & Holw. Mycologia **23**: 333. 1931.

Pycnia subcuticular, amphigenous or caulicolous. Aecia and uredia wanting. Telia amphigenous, caulicolous or petiolicolous, small or becoming confluent, on hypertrophied areas, chestnut-brown, pulverulent, paraphyses few, discal or peripheral, cylindric, thin-walled; teliospores (PLATE 16, FIG. 9, 10) borne two (rarely 3) on a pedicel, oblong or ellipsoid, 12–16 μ wide by 22–28 μ high; wall 1–1.5 μ thick, cinnamon-brown, verrucose with cubical projections, more abundant above, pore one, apical, at the inner angle; pedicel hyaline, with two apical cells, fragile and breaking below the apical cells.

HOSTS AND DISTRIBUTION: *Inga* sp., in Brazil.

TYPE LOCALITY: Petropolis, Rio de Janeiro, Brazil, on *Inga* sp.

ILLUSTRATIONS: Jackson, in Mycologia **23**: 334. 1931, *fig. 1.*

EXSICCATI: Reliquiae Holwayanae *281.*

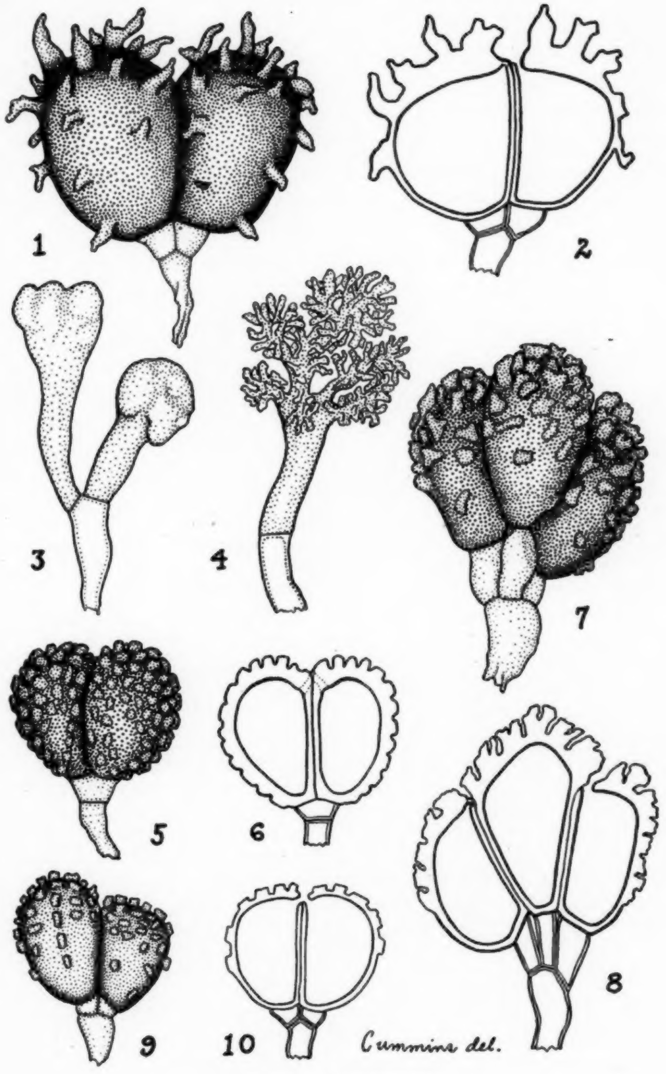
Rarely three spores can be found borne by one pedicel.

As published by Jackson the generic name reads *Dichaerina* but is here corrected to accord with Arthur's spelling, *Dicheirinia.*

4. **Dicheirinia manaosensis** (P. Henn.) comb nov.

Diorchidium manaosensis P. Henn. Hedwigia **43**: 159. 1904.

Pycnia subcuticular. Aecia and uredia wanting. Telia subepidermal, deep seated, without paraphyses, chestnut-brown, borne on hypertrophied spots on the leaves and stems; teliospores (PLATE 16, FIG. 7, 8) obovoid, 15–20 μ wide by 25–33 μ high, borne 3 (rarely 2) on a pedicel; wall 1.5–2 μ thick, chestnut-brown, coarsely verrucose with cubical or digitate projections, more abundant above, often in rows on the sides and wanting below, pore one, apical and near the inner angle; pedicel hyaline, with three apical cells, short and fragile.



SPECIES OF DICHEIRINIA

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HOSTS AND DISTRIBUTION: *Lonchocarpus rariflorus* Mart., in Brazil.

TYPE LOCALITY: Rio Negro, Manáos, Brazil on *Lonchocarpus rariflorus* Mart.

A microcyclic species characterized by its teliospore pedicel with three apical cells each bearing a teliospore. Hennings (*l. c.*) was in error in describing the species as having 2-celled teliospores and made no mention of the structure of the pedicel.

While one spore is commonly somewhat higher than the other two it has its own pedicellate cell and cannot be considered comparable to *Hapalophragmium*. This addition to the 2-spored condition typical of *Dicheirinia* may indicate a tendency toward spore-heads as in *Ravenelia*. *Ravenelia simplex* Diet. offers a close parallel in development.

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EXPLANATION OF PLATE 16

All figures were drawn with the aid of a camera-lucida and represent an enlargement of approximately 625 diameters.

Fig. 1, *D. binata,* a perspective drawing of the teliospores to show the peculiar sculpturing of the wall and the structure of the pedicel; 2, *D. binata,* an optical section of the teliospores giving wall thickness and pore location; 3, *D. binata,* refractive, clove-like paraphyses found in the aecia, uredia and telia; 4, *D. Ormosiae,* a botryoid paraphysis as seen in the uredia and telia; 5, *D. Ormosiae,* perspective drawing of the teliospores showing the cubical wall-sculpture and the single apical cell of the pedicel; 6, *D. Ormosiae,* optical section of the teliospores indicating wall thickness and probable location of pores; 7, *D. manaosensis,* perspective drawing of the teliospores to show spore sculpture and pedicel; 8, *D. manaosensis,* optical section of the teliospores; 9, *D. superba,* perspective drawing of the teliospores showing spore sculpture and the two apical cells of the pedicel; 10, *D. superba,* optical section of the teliospores to show the thin wall and location of the pores.

NEW OR LITTLE KNOWN CHYTRIDIALES[1]

JOHN N. COUCH

(WITH 64 TEXT FIGURES)

During the springs of 1923, 1924, and 1925 a fungus was observed to be parasitic within the threads of *Pythium gracile* and *P. dictyosporum,* the two species of *Pythium* being in turn parasitic in the threads of *Spirogyra areolata* and *Spirogyra* sp. In the development and structure of the plant body the fungus showed a striking resemblance to certain species of *Olpidiopsis;* in the development and behavior of the spores it resembled *Aphanomycopsis* Scherffel and certain species of *Ectrogella* (sense of Scherffel), while in sexual reproduction there was a resemblance to *Pythium.* This peculiar combination of characters separated this fungus from any other previously described and therefore it seemed logical to erect a new genus combining some of the characters of each of the following genera: *Olpidiopsis, Ectrogella, Aphanomycopsis,* and *Pythium.*

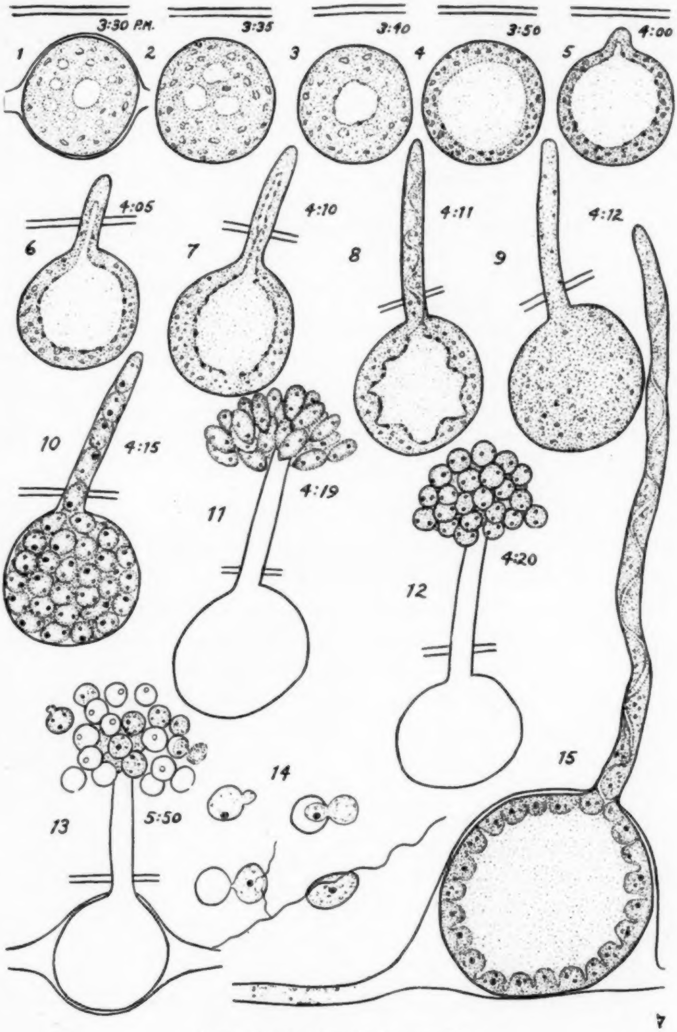
Pythiella gen. nov.

Plant body parasitic within the threads of *Pythium;* without rhizoids, the entire thallus upon maturity being transformed into reproductive organs. Spore development as in the higher water fungi (*Achlya* and *Saprolegnia,* e.g.). Spores after emergence encysting at the tip of the sporangium as in *Achlya,* swarming later in the laterally biciliate condition. Antheridia present on all oögonia. Egg not completely filling the oögonium, and with a distinct periplasm.

Pythiella vernalis sp. nov. (TEXT-FIGURES 1–27).

Sporangia developing in the threads of *Pythium,* spherical or rarely subspherical when mature, without mycelium and rhizoids; causing the formation of a distinct gall in the *Pythium* thread, usually one sporangium in each gall though 2, 3, or 4 may not

[1] Presented before the Mycological Society of America, 1932.



FIGS. 1–15. *Pythiella vernalis.*

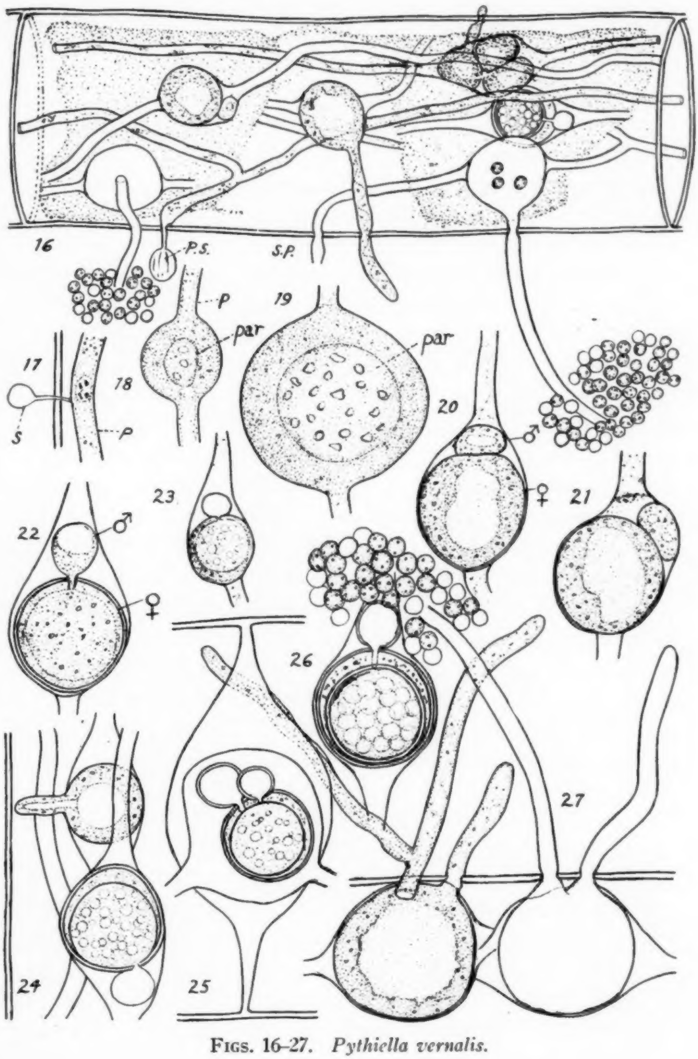
uncommonly occur; 10–30 μ thick, emptying through a long tube up to 50 μ long and about 4 μ thick; on some sporangia several tubes may be formed (as many as five), some of which may be branched. Spores diplanetic, encysting, after discharge, at the tip of the sporangial tube, emerging from the cysts after about an hour, elongated with a longitudinal groove and with two cilia; spores 3.7–4 μ thick. In swimming the active cilium is directed forward while the posterior one is dragged along behind. Sexual reproduction is by oögonia and antheridia; oögonia 11–18.5 μ thick, spherical, containing one egg, which does not completely fill the oögonium; eggs 9–15 μ thick, spherical, when mature surrounded by a thick wall and a small amount of periplasm; antheridium spherical, about 5 μ thick, emptying its entire contents through a delicate tube into the egg.

Collected several times during the late spring, 1923, 1924, and 1925. Type represented by preserved material on slides in the University of North Carolina Herbarium. Growing as an endophytic parasite within the threads of *Pythium gracile* and *P. dictyosporum*, the two species of *Pythium* being in turn parasitic in the threads of *Spirogyra areolata* and *Spirogyra* sp.

DEVELOPMENT OF SPORANGIA

The spore comes to rest on or near the *Spirogyra* thread, sprouts a fine tube which first penetrates the *Spirogyra* wall and then the *Pythium* thread to discharge its contents into the latter. The empty spore-cyst remains visible on the *Spirogyra* thread for some time after infection. Within the *Pythium* thread, the parasite assumes a rounded or oval shape. It appears to be surrounded by a definite wall and its protoplasm contains numerous, large, glistening granules which are considerably larger than the granules in the *Pythium* thread. As the parasite develops, the host becomes swollen in the region of the thread immediately around the parasite, forming a gall much like that formed in the Saprolegniaceae by *Olpidiopsis*. As a rule each gall contains only one sporangium but I have seen several which contained 2, 3, or sometimes even 4 sporangia, and in such cases the sporangia are more or less flattened by mutual pressure.

It is sometimes quite difficult to determine whether the parasite is within the *Pythium* thread or is merely loose in the *Spirogyra* cell. This difficulty is increased where the gall is partly obscured by the disintegrating contents of the *Spirogyra* cell. Fortunately



FIGS. 16–27. *Pythiella vernalis.*

the material was very abundant and by finding places where the contents of the *Spirogyra* cell had mostly disintegrated it was possible to determine beyond doubt that the galls are within the *Pythium*. A number of cases were seen where the *Pythium* thread, still connected with the old, infecting spore-cyst, contained several of the parasites. Other cases were seen where a thread connected with a sporangium contained one of the parasitic bodies. Again several places such as that shown in figure 25 were seen. Here the gall containing an oögonium and two antheridia has developed in the *Pythium* thread between the two parts of the cross wall of the *Spirogyra* cell. Sometimes the *Pythium* threads may extend from one *Spirogyra* thread through the water to another thread. I have never observed such threads attacked by the parasite. However, I have observed numerous sporangia and sexual bodies of the parasite lying in trash in the water apparently unconnected with the threads of the *Pythium* or *Spirogyra*. If one looks closely, however, he may detect the disintegrated remains of the *Pythium* and *Spirogyra* threads.

Unfortunately no satisfactory tests were made to determine the chemical composition of the cell wall of the parasite while the material was fresh. Some of the material preserved on a slide in glycerine in 1925 was washed and tested (May 1933) with chlorzinc iodide. The *Spirogyra* and *Pythium* walls, where the latter were exposed, gave a beautiful blue reaction but the parasites in the *Pythium* were uncolored by the reagent even where the tubes were projecting out through the *Pythium* and *Spirogyra* walls. This test was performed on two lots of material with results as indicated above and, while not entirely convincing, it suggests strongly that the wall membrane of the parasite is not pure cellulose but rather has a composition similar to that of the Chytridiales.

Shortly before the sporangium attains its mature size the large granules disappear and the protoplasm, except for several small vacuoles, becomes homogeneous. After the sporangium becomes mature in size the development of the spores proceeds rapidly. The small vacuoles flow together to form a single, large, central one. The pressure within this vacuole appears to increase so that the protoplasmic layer becomes quite thin. Meanwhile the tube for the emergence of the spores is formed. As this tube grows

the vacuole extends out through its center. The protoplasm, both in the sporangium and in the tube, becomes arranged in numerous irregular heaps as the furrows of the vacuole push outward toward the cell wall. This stage corresponds to the " spore initial " stage in the Saprolegniaceae and the " balling " stage in *Ectrogella* and *Aphanomycopsis.*

The vacuole pushes outward until suddenly it breaks at one or more places and almost immediately the protoplasm appears to occupy the entire space in the sporangium. This change appears to be accompanied by a decrease in the size of the sporangium. This stage corresponds to the " homogeneous " stage in the development of the sporangium in the Saprolegniaceae.

In a very few minutes (3–5) after the " homogeneous " stage, the completely formed spores appear. The spores are not equipped with cilia and appear motionless within the sporangium. The pressure within the sporangium increases so that the tip gives way and suddenly the spores rush out with great rapidity. Usually all the spores emerge but sometimes a number may be left in the sporangium to encyst there. Immediately after the spores emerge, they are elliptic and somewhat pointed at the bases which seem to be connected with the tip of the emergence tube. The spores retain this shape for only a few seconds, quickly rounding up and encysting in the same position. Each spore possesses two or more conspicuous granules.

After remaining quiet at the sporangial tip for some time (usually one to two hours) the spores emerge from their cysts. This process is as in *Achlya* or *Saprolegnia.* The zoöspore is elongated, ovoid, with a median groove from which arise two cilia. As the spore swims the active cilium is directed forward while the posterior cilium is dragged along behind. The movements of the spores through the water are smooth, the spore describing a spiral path as it moves forward and also rotating on its axis, the movement being like that described by Weston (1918) for the zoöspores of *Thraustotheca clavata.*

DEVELOPMENT OF THE OÖGONIA AND ANTHERIDIA

Although I have observed a large number of stages in the development of the sexual organs of this fungus, I have not followed

through their development. Each oögonium is accompanied by a much smaller antheridial cell and not rarely there may be two of the male cells applied to one oögonium. Both cells are contained within the host thread. After the oögonium reaches its mature size one may recognize a large irregular vacuole. This increases in size, the parietal protoplasm being simultaneously thinned down in a few places and heaped up in others. The number of "heaped-up masses" in the oögonium at this time is many fewer than in the sporangium when the spore origins appear. I have not followed the ultimate fate of the vacuole but it continues to push outward, the parietal protoplasm becoming thinner and thinner at certain places. At a later stage the contents of the oögonium are divided into a dense central spherical mass and a thinner peripheral layer of protoplasm. Although the nuclear details have not been worked out, it appears that the inner spherical mass is an egg and the outer layer is periplasm. The antheridium sends a delicate tube through the oögonial membrane and periplasm into the egg and into this discharges its entire contents. As the egg matures, its wall becomes considerably thickened and all or nearly all of the periplasm disappears. Presumably this takes part in the thickening of the egg wall. I have not observed the germination of the eggs.

RELATIONSHIPS

This fungus is apparently very closely related to *Ectrogella* in the sense of Scherffel and to *Aphanomycopsis* Scherffel. The genus *Ectrogella* was established by Zopf (**1884**) on the single species *E. bacillariacearum* but Scherffel (**1925**) has added several new species and has brought to light new information which indicates a close relationship between this genus and the Ancylistineae and Saprolegniaceae-Peronosporaceae series rather than to the Chytridiaceae series. In *Ectrogella* the sporangia have a central vacuole. A "balling stage" occurs. The zoöspores usually have two cilia and swim smoothly. These characters belong to the former series rather than to the Chytridiaceae. Scherffel was the first to call attention to the difference in the appearance of the protoplasm in the vegetative threads of the Saprolegniaceae and the cells of the Chytridiales and the Ancylistales, the protoplasm in the former having a granular consistency while in the

latter two orders it has a pale whitish fat-gleam. This difference is striking and can easily be observed in *Myzocytium* and *Lagenidium* (probably also in *Ancylistes,* though I have not noticed it). The protoplasm of *Ectrogella* and *Aphanomycopsis* is like that in the Saprolegniaceae and therefore Scherffel thinks them to be more closely related to the latter order than to the Ancylistales.

While the present species is close to certain species of *Ectrogella* (*E. monostoma*) and *Aphanomycopsis* in spore development and behavior, it differs from them both in the appearance of the protoplasm, for here it resembles that of *Myzocytium, Lagenidium,* and *Olpidiopsis* in having a pale, whitish gleam and in containing a few, large, conspicuous, irregular, glistening granules. Moreover, the spores of *Aphanomycopsis* are almost twice as large as in the present species.

In sexual reproduction the present species is distinct from *Aphanomycopsis* and *Ectrogella.* In *Aphanomycopsis,* according to Scherffel (**1925**), the resting spores are like the oöspores of *Saprolegnia* in structure, but arise asexually in a rudimentary oögonium. In one species of *Ectrogella, E. Licmophorae,* resting spores are formed and these arise by a sexual act, apparently much as in *Myzocytium proliferum* (**Scherffel, 1925**).

The present species may easily be distinguished from *Myzocytium, Lagenidium,* and *Achlyogeton,* to each of which, however, it shows certain similarities in structure which perhaps indicate homoplasmy rather than genetical relationship. It may also be distinguished from *Achlyella,* an imperfectly known genus described by Lagerheim (**1889**), by the extramatrical sporangia of the latter. The two genera agree in the encystment of the spores at the sporangial tip. In *Achlyella,* however, the size, shape, number of cilia, and method of swimming of the spores are not known, and therefore a satisfactory comparison of these two genera must wait until *Achlyella* can again be found and adequately described. It also resembles *Olpidiopsis Schenkiana* Zopf in sexual reproduction, but may be distinguished from that species by spore behavior and the absence of periplasm in the oögonia of *O. Schenkiana.* Furthermore, the chemical composition of the cell wall in *Olpidiopsis,* which gives a beautiful purplish reaction with chlorzinc iodide, would exclude the present species from that genus.

This species shows a superficial resemblance to *Pseudolpidium Aphanomycis* (Cornu) Fischer as described and illustrated by Butler (**1907**). The galls, the shape of the sporangia, and their emergence tubes are quite similar, but the spore behavior in the two genera is markedly different.

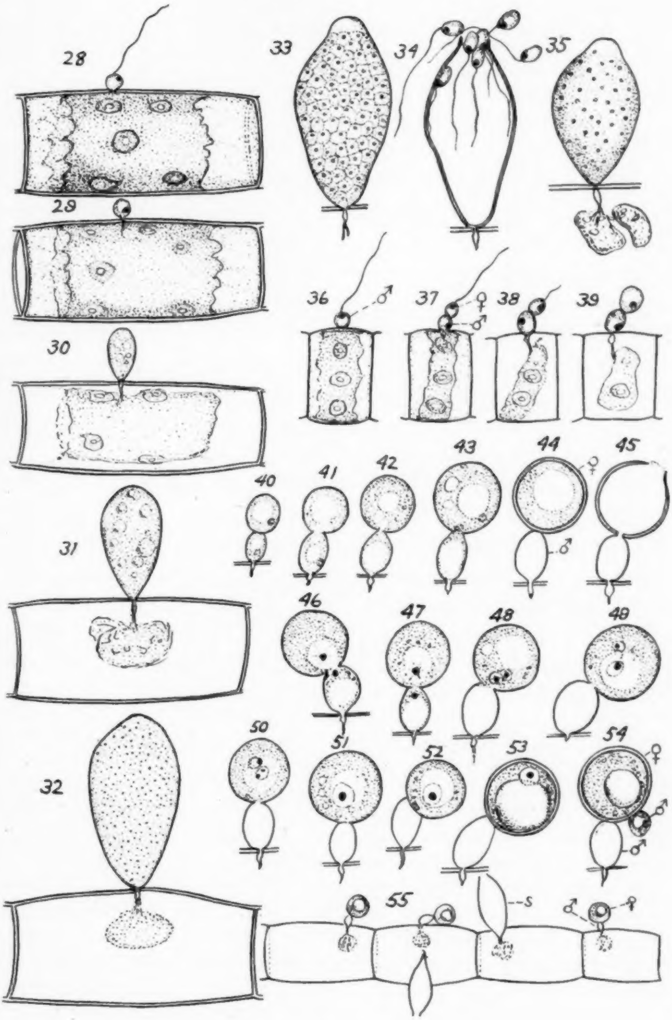
The following species of *Rhizophidium* has appeared only once. Its peculiar type of resting spore formation, in which the female gamete is the more active and searches out the male, perhaps justifies its presentation here.

Rhizophidium ovatum sp. nov. (TEXT-FIGURES 28–55).

Mature sporangia pear-shaped or oval, thickest in the distal half; attached to the host by a minute bulbous swelling from which arises a very slender rhizoid which extends to the chromatophore of the host. Sporangia 8.4–16.8 × 16–30 μ, most about 13 × 20–25 μ. Spore formation as in the genus. Sporangial dehiscence apical. The tip of the sporangium gelatinizes, suddenly bursts and the spores emerge with great rapidity, the sporangium becoming empty in a few seconds. Spores swimming away upon emerging, slightly elongated, about 3 × 4 μ, with a large oil globule and a long posterior cilium. Spores swimming by a darting and hopping motion. Sexual reproduction of a peculiar type: the male cell settling down on the host, penetrating the latter to develop the bulbous swelling and minute rhizoid; the female cell later attaches itself to the male. Now fed indirectly from the host through the male, the female increases rapidly in size; the male cell increasing in size considerably or not at all, but finally emptying its entire contents into the female. Male cells or antheridia usually spherical or slightly subspherical, 3.6–5 μ, most about 4.4 μ thick; often oval and then usually larger, such antheridia being up to 5 × 7 μ; female cells, or oögonia, when mature, 5.4–9.6 μ, most about 8.4 μ thick, spherical, when ripe with a slightly thickened wall and a single, large, slightly excentric oil globule. Oögonia germinating after a rest period of two or three days to form spores.

Found only once, May 6, 1931, on *Stigeoclonium* (?) sp. in small wet weather pond on way to Laurel Hill, Chapel Hill, N. C.

This species apparently belongs to the genus *Rhizophidium*, though because of the minute bulbous base and the very fine rhizoid which is very difficult to see one might put the species in the genus *Phlyctidium*. In fact the rhizoid was so fine that I could



FIGS. 28–55. *Rhizophidium ovatum.*

never be absolutely certain if there were only one rhizoid or several.

In asexual reproduction the present species is close to *R. Fusus* (Zopf) Fischer and *R. Lagenula* (A. Br.) Fischer. In *R. Fusus* a very extensive rhizoidal system is developed, while in the present species the rhizoid or rhizoids are very minute. Both fungi have elongated sporangia but in *R. Fusus* the sporangia are thickest in the middle while in *R. ovatum* they are thickest in the distal half. In *R. Fusus* the resting spores have not been observed. *Rhizophidium Lagenula* may be easily separated from *R. ovatum* by the fact that the former is slightly longer and much thinner than the latter.

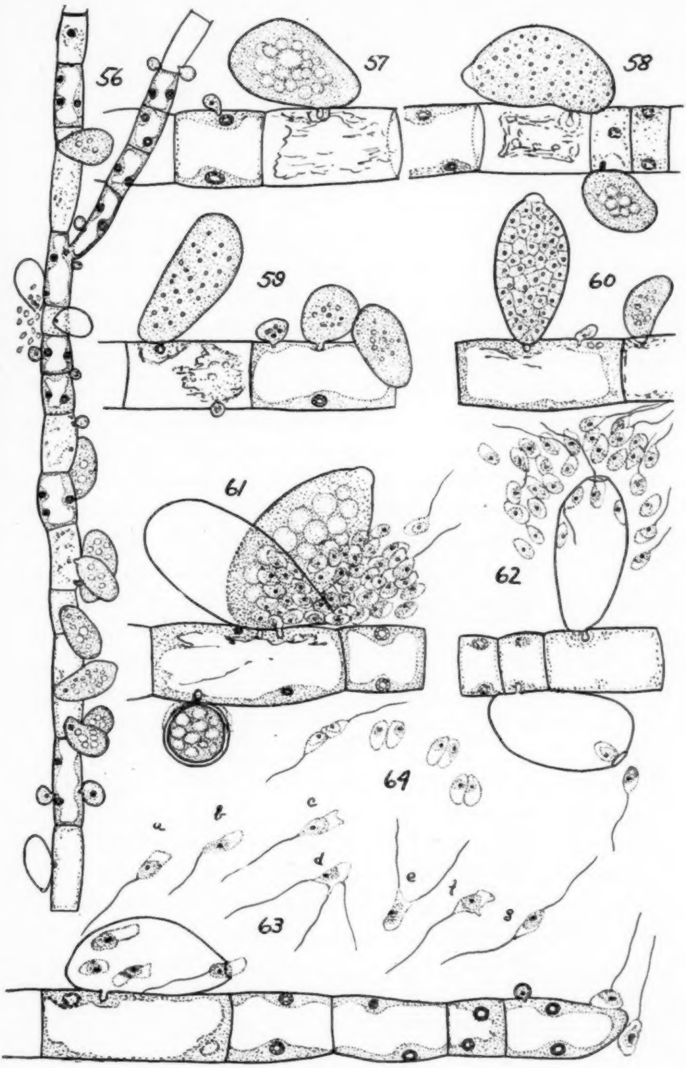
In sexual reproduction the fungus appears to resemble *R. granulosporum* Scherffel. In this species Scherffel (**1925b**) shows the mature zygote attached to the smaller, empty, male gamete rather than to the host cell. The male gamete is attached to the host cell as in the present species. The wall of the zygote in *R. granulosporum* Scherffel is spiny while in *R. ovatum* it is smooth. Moreover, the shape of the sporangia in the two species is quite distinct.

I am unable to say whether the gametes are borne in the sporangia along with the zoöspores or are formed in separate gametangia.

Fixed material stained with Gram's gentian violet showed that the gametes are uninucleate and that the nucleus from the smaller, male cell passes along with the cytoplasm over into the female cell, fusing with the nucleus of the latter.

Phlyctidium anatropum (Braun) Sparrow (TEXT-FIGURES 56–64).

Sporangia attached to the filaments of *Stigeoclonium* by means of a small rounded haustorium; very irregular in shape, usually ovoid and flattened on one side, often attached on the flattened side, sometimes ovoid and symmetrical; $9–14 \times 16–25\ \mu$. Spore development somewhat as in *Rhizophidium*. As the sporangium matures a hyaline papilla is formed on one end of the sporangium. When the spores are mature, the sporangial wall gives way at this point and the spores emerge. Spores about $2.1 \times 5\ \mu$; encysted spores subglobose, about $2.2–3\ \mu$ thick, with a small, inconspicuous dot or fat globule. The first spores emerge more rapidly than the later ones though they all come out with characteristic slowness. As a rule they hover around the sporangium for some time



FIGS. 56–64. *Phlyctidium anatropum.*

after emerging. Spores uniciliate, but the cilium is apparently functionless since the spores move in an amoeboid fashion dragging the cilium along behind. Spores frequently forming one or several hair-like pseudopodia. Uniciliate gametes are formed which unite in pairs, perhaps fusing, the zygote apparently developing into a thick-walled, spherical, resting body about 10 μ in diameter.

Easily recognized by the asymmetrical sporangia and by the uniciliate, amoeboid zoöspores.

On *Stigeoclonium* sp., Chapel Hill, N. C., May 15, 1931; also collected by Sparrow on *Stigeoclonium* sp., Bessemer, N. Y., January 1932.

When this organism was first seen by me, I took it for encysted stages in some peculiar protozoan. It was not until its development was followed and sporangia in the act of spore discharge were observed that its true nature was suspected. The sporangia have a rather unique appearance in the stages preceding the beginning of spore formation. This is not shown sufficiently distinctly in any of my figures except perhaps the larger sporangium in figure 57 and the smaller one in figure 58. There is a thick, clear, outer region of protoplasm enveloping the inner region of fat globules. When the time for spore formation approaches, the globules of fat (?) are digested and numerous minute globules are evenly distributed throughout the protoplasm (FIG. 58 AND 59). As the spores are formed, each one contains a single, small globule but these bodies are not so conspicuous as in the spores of *Rhizophidium*.

By isolating material early in the morning and making frequent observations throughout the day, it was found that the spores are usually discharged between 6 and 8 o'clock in the evening. Spore discharge was observed about the same time on several successive evenings, occurring about the same time in the original material in a Petri dish as in material isolated on slides. The first spores to be discharged and by far the greater number are apparently forced out by some internal pressure, the cilium being dragged behind as they pass through the opening. The later spores creep out by amoeboid motion. Due to the inactivity of the cilia the spores remain for several minutes in a loose cluster at the tip of the sporangium and then slowly creep away. The spores creep over the surface of the threads and over the slides, the movement

keeping up for over an hour but in no instance have I observed any other motion than the amoeboid type. The spores are elongate and are composed of two distinct regions: a region of granular protoplasm to which the cilium is attached and a hyaline zone, which makes up about half the spore, opposite the ciliary end. It is this hyaline zone which changes shape, producing at times short pseudopodia (FIG. 63c) or at other times long hair-like ones (FIG. 63d).

Spores or gametes were frequently observed grouped in pairs but I could not be positive about their final fusion (FIG. 64). My observations were quite similar to those of Ledingham on a species of *Rhizophidium* (in paper given before the Mycological Society of America, Christmas meeting, 1932), who not only observed the fusion of the gametes but also observed that the zygote formed resting sporangia. After the Christmas meetings, I looked over my slides again and found a considerable number of thick-walled resting sporangia attached to the host threads with similar, minute, bulbous haustoria as found on the zoösporangia.

The present fungus appears to be identical with Sparrow's (**1933**) description and figures of *Phlyctidium anatropum* (Braun) Sparrow. Sparrow's fungus, like mine, occurred on *Stigeoclonium* sp. and agrees in nearly all details. He did not observe spore discharge, in fact, if the species is correctly identified, this is the first time spore discharge has been observed. I have not seen Braun's original paper but, according to Sparrow, Braun observed thick-walled ovoid resting sporangia. In the genus *Phlyctidium*, several species of which I have studied, the spores are spherical with a single large globule, and uniciliate, the movement of the spores being characteristically of the chytridiaceous type and not amoeboid. The peculiar amoeboid movement of the spores with the formation of the hair-like pseudopodia would seem to separate the present species rather sharply from the genus *Phlyctidium*. Since the synonymy of this species is already rather complicated it would be better to wait until its complete life history is certainly known before removing it from *Phlyctidium*.

The damage done to the algal host is not so noticeable in fresh material as in material preserved in glycerine. However, as a rule, even in fresh material one can see that the protoplasts of the host are disorganized as the parasite develops. In preserved material

the injury is more noticeable, due to the greater plasmolysis of the parasitized cells.

SUMMARY

A new genus containing one species (*Pythiella vernalis*) is described which is related to *Ectrogella, Aphanomycopsis,* and *Olpidiopsis Schenkiana.* The fungus is parasitic in the threads of *Pythium gracile* and *P. dictyosporum* which are in turn parasitic on the threads of *Spirogyra areolata* and *Spirogyra* sp. In asexual reproduction the fungus resembles *Ectrogella* while the presence of periplasm in the oögonium suggests a remote relationship to *Pythium.* The protoplasm of *Pythiella* has the pale whitish gleam and the fat globules characteristic of the Chytridiales and Ancylistales whereas in *Ectrogella* the protoplasm has a granular appearance as in *Saprolegnia.* It would seem that *Pythiella* should be placed in the Chytridiales close to *Olpidiopsis Schenkiana* (sense of Butler and Scherffel).

A new species of *Rhizophidium, R. ovatum,* parasitic on *Stigeoclonium,* is described. It is characterized by the ovate sporangia, the very minute rhizoids, and the peculiar type of sexual reproduction in which the female gamete is the more active. The gametes are uninucleate. The contents of the male cell pass into the female and the two nuclei unite. The zygote after a short period becomes a zoösporangium.

Phlyctidium anatropum (Braun) Sparrow is described. The organism is recognized by the asymmetrical sporangia and by the uniciliate but amoeboid zoöspores. The latter are described for the first time. Uniciliate gametes (?) which unite in pairs, perhaps fusing, to form a thick-walled, spherical, resting body are described.

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EXPLANATION OF FIGURES

(Figures inked in by Miss Alma Holland)

Fig. 1–27. *Pythiella vernalis* Couch (1–14, illustrate continuous observations on one single sporangium). 1–4, show coalescence of small vacuoles to form a large central vacuole; 5–7, show formation of emergence tube; 8, appearance of spore origins; 9, disappearance stage; 10, reappearance of spores; 11, spores immediately after discharge and before encystment; 12, spores encysted; 13, spores emerging from cysts one hour and thirty minutes later; 14, more highly magnified view of spores emerging from cysts and showing cilia; 15, section view of large sporangium showing stage just before disappearance of spore origins; 16, habit sketch showing parasites within *Spirogyra* cell. Note disintegrating chloroplasts; *Pythium* threads in which are asexual and sexual stages of *Pythiella;* also empty spore cyst of *Pythium* (p.s.) and empty sporangium of *Pythium* (s.p.); 17, early stage of infection. s, empty cyst of *Pythiella;* p, *Pythium* thread; 18 and 19, development of the gall. p, *Pythium* thread; par, *Pythiella.* Note granules of fatty substance in protoplasm of latter; 20–26, stages in the development of oögonia and antheridia. Note periplasm in figures 22–26. Figure 25 shows parasitic gall formed in *Pythium* thread between end walls of two *Spirogyra* cells; 27, two sporangia with several emptying tubes, one of which is branched; All above figures × 1350 except figure 16 about × 675 and figure 14 about × 2000.

Fig. 28–55. *Rhizophidium ovatum* Couch. 28–34, stages in development of one single sporangium from infection by spore 7:30 A.M. to 3:27 P.M. the following day. Note disintegration of chloroplast; 35, sporangium a few hours before spore discharge; 36–44, development of zygote, living material; 45, zygote which has formed a sporangium; 46–53, killed and stained material showing development of zygote; 54, zygote with two male cells; 55, habit sketch showing sexual and asexual reproductive bodies. Fig. 28–54 × 1250; fig. 55 × 620.

Fig. 56–64. *Phlyctidium anatropum* (Braun) Sparrow. 56, habit sketch showing numerous parasites on thread of *Stigeoclonium;* 57–60, sporangia in various stages of development. The large sporangium in figure 57 and the smaller one in figure 58 show thick, peripheral, hyaline zone of cytoplasm surrounding central region of globules. Larger sporangia, figure 58 and 59, show minute droplets, one for each spore; 61–63, sporangia with spores in the act of emerging. Note how spores hover around sporangial mouth, figure 61 and 62. In figure 63 the letters show successive changes in the same spore as it was watched during a period of several minutes. In figure 61 one resting sporangium is shown; 64, gametes in act of fusing. Four pairs are shown. Many such were seen. Fig. 56 × 620; others × 1250.

SOME NON-CATENULATE CONIDIAL PHYCOMYCETES PREYING ON TERRICOLOUS AMOEBAE

CHARLES DRECHSLER

(WITH 5 TEXT FIGURES)

In a summary (**3**) published in 1933 were set forth briefly the morphological features of five fungi that had been found capturing and killing *Amoebae* in aging agar plate cultures started from plantings of diseased rootlets and other decaying vegetable materials. The continuous mycelium in four of the five forms (**3**, *figs. 2–5*) obviously characterized them as Phycomycetes, as did also the direct origin of the subspherical sexual spores through fusion of paired filaments figured for three of the species (**3**, *figs. 3–5*). No definite assignment of the fungi was then attempted, partly for the reason that the meager differentiation of the fusing elements, together with the extraordinarily small dimensions of the sexual apparatus, introduced serious difficulties of interpretation. Moreover, though the study of the asexual reproductive phase entailed less optical uncertainty, the conidia showed no close parallelism in structure or in manner of origin to those of any of the better known groups in the Phycomycetes, being suggestive rather of types known among the Mucedinaceae especially with respect to shape and, in two of the species, to the presence of empty appendages.

More recently the four minute non-septate predacious forms were discussed (**6**) as members of a new major group of Phycomycetes for which the term Zoopagaceae was suggested as being a tolerably appropriate one. The fairly unambiguous morphology of the several remarkable endoparasitic, ectoparasitic and predacious species about which more particularly the new group was integrated, furnishes now a background for a more satisfactory description of the minute *Amoeba*-capturing organisms than could have been undertaken earlier. In addition to the four taken up in the summary, an equal number of closely related predacious

species that later came under observation are included for discussion. All eight are represented in their vegetative phase by an extensive mycelium on which terricolous *Amoebae*, mostly of the smaller and medium sizes, are captured through adhesion. The general biological relationship is thus broadly comparable to that described previously in the account of *Zoopage phanera* Drechsl., though the endozoic parts, instead of constituting a distinctive compact haustorium, make up a branching system only little differentiated from the mycelium generally.

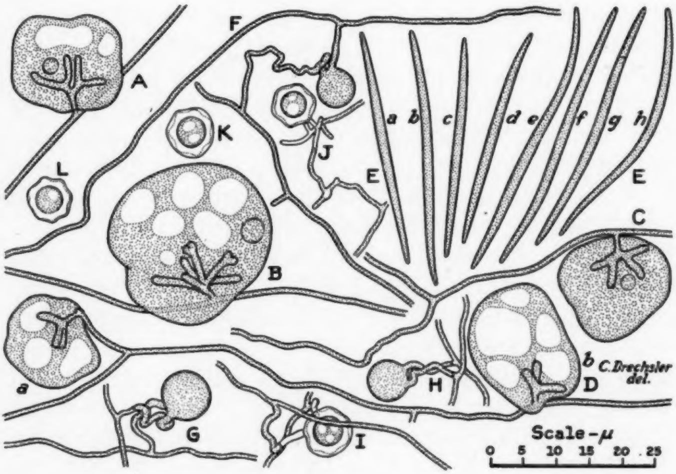

FIG. 1. *Acaulopage rhapidospora.*

If the conidia of the species herein described are hardly to be regarded as of large size in comparison with those of fungi generally, they are yet of generous dimensions when viewed in relation to the delicate mycelia and the minute sexual structures associated with them. An analogous proportionality in size obtains in the predacious *Zoopage phanera,* but not in any of the related parasites, internal or external, assigned (**6**) to the genera *Endocochlus, Cochlonema* and *Bdellospora,* even though the latter two genera share in the catenate development of asexual spores represented in *Zoopage.* The explanation of these size relationships lies very

probably in the requirements entailed in a predacious as contrasted with a parasitic habit. A conidium designed to be ingested by its *Amoeba* host manifestly needs to be no larger than of a size just sufficient to start a new thallus. The production of a short germ tube and of a minute thallus later to be detached, exemplified in the development of the conidium of *Endocochlus asteroides* Drechsl., likewise requires only a moderate expenditure of protoplasmic substance. Nor does the proliferation of an incipient haustorium by the conidium of *Bdellospora helicoides* Drechsl., preceding the instigation of autonomous development, require any great outlay of material. On the other hand, an organism dependent for its existence on the capture of animals as minute and as slow of movement as the smaller *Amoebae*, and besides present often only in rather small number, would obviously have need from the very beginning of a fairly extensive mycelium. The interception and capture of prey under conditions at all difficult would obviously require beforehand a rangy development of germ hyphae from the substance of the conidium itself (FIG. 5, F).

Because of their dimensions and the frequency with which they make their appearance on old isolation plate cultures, the conidia of the fungi under consideration can hardly have escaped being seen by mycologists at least occasionally in the course of routine operations. That they have evoked little if any comment is very probably to be attributed in part to their mostly commonplace appearance, and in part to the difficulty of determining the manner in which they are borne. Thus, for example, the very distinctive conidia produced by the species now to be described as *Acaulopage tetraceros* have for many years put in appearance from time to time in my own cultures, but their haphazard distribution on the substratum and the absence of anything recognizable as conidiophores, uniformly gave the impression that they represented spores of some *Tetraploa*-like hyphomycetous form that had been introduced accidently and been scattered about through disturbances incident to microscopic examination. Similarly the sexual apparatus of some of the forms described herein are objects that have been long familiar to me in old isolation plate cultures, but since the hyphae supporting them become almost wholly invisible after being evacuated, they were confused with the angular cysts of

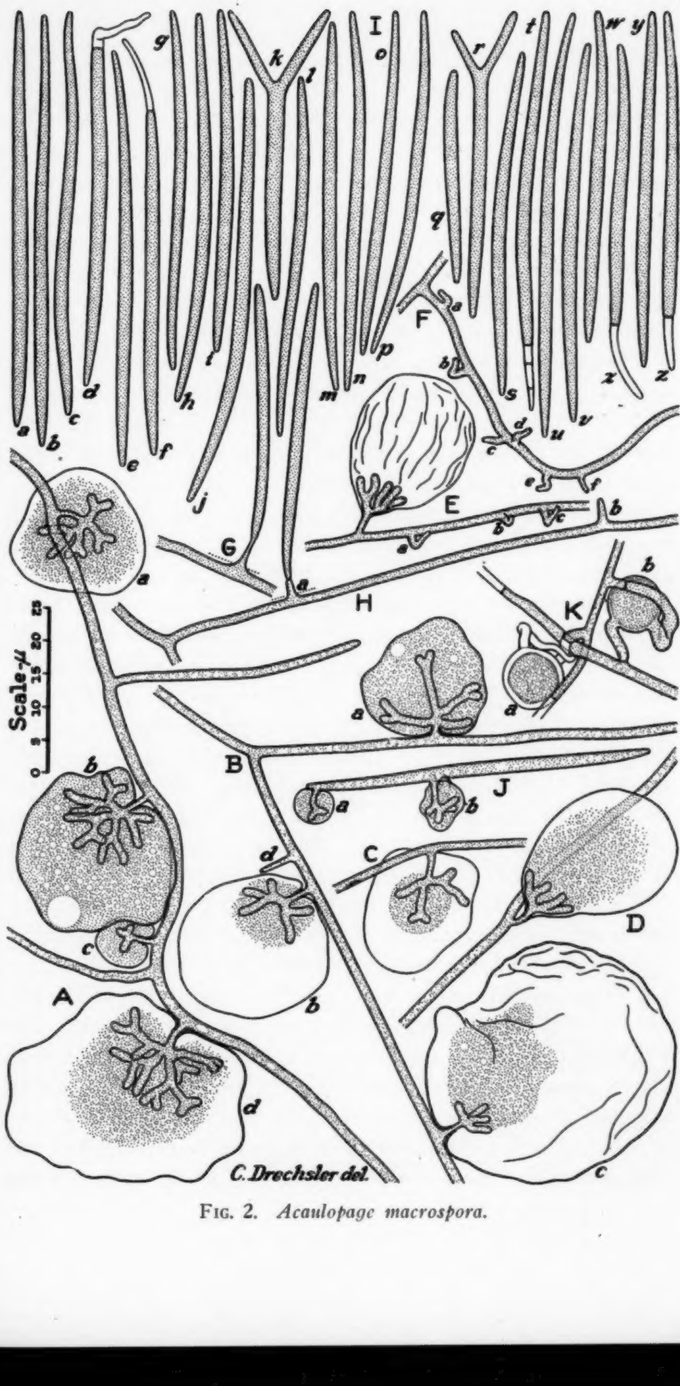

FIG. 2. *Acaulopage macrospora.*

some small protozoans habitually and abundantly present in such preparations.

Undoubtedly, too, the very fact that predacious fungi ordinarily make their appearance in an isolation plate culture only after it has been overrun by various much more luxuriant and conspicuous saprophytic forms, has helped to keep them in obscurity. This tardiness in making their appearance is due evidently not so much to the generally somewhat slow rate of growth of the fungi themselves as to the time required for conditions to arise permitting any development of them at all. Conidial apparatus is usually not produced in such quantity as might invite notice until the underlying vegetative mycelium has attained some extension. Since all members of the group are apparently entirely dependent for their nourishment on animals, extensive mycelial growth of the predacious forms can not take place until prey is present in quantity. However, an abundant supply of *Amoebae*, or for that matter, of nematodes, is usually not available until the relatively few living specimens in the piece of decaying vegetable material from which the culture was started, have multiplied for a week or two. There is evidence indicating that the animals in question feed on the bacterial slime and fungous spores present on the cultures, rather than directly on the agar substratum; so that the establishment first of a congenial fungous and bacterial flora, and then of a suitable fauna of microscopic invertebrates, needs to precede any noticeable development of predacious types. As might be expected under the circumstances, predacious forms unlike many of the common Phycomycetes, show no mycelial degeneration from contact with bacterial slime.

The character of the rapidly growing fungi first extending themselves over an agar plate, in influencing the trend of subsequent development of a subsidiary microflora, and thereby the composition of the infesting microfauna, affects greatly the abundance and identity of the predacious Phycomycetes appearing later, just as it affects greatly the abundance and identity of the predacious Hyphomycetes. Fungi with a dense dry aerial mycelium, or with sporophores in dense arrangement, as, for example, species of *Mucor, Rhizopus, Penicillium, Alternaria* or *Hormodendron,* not only give little encouragement to bacterial development, but im-

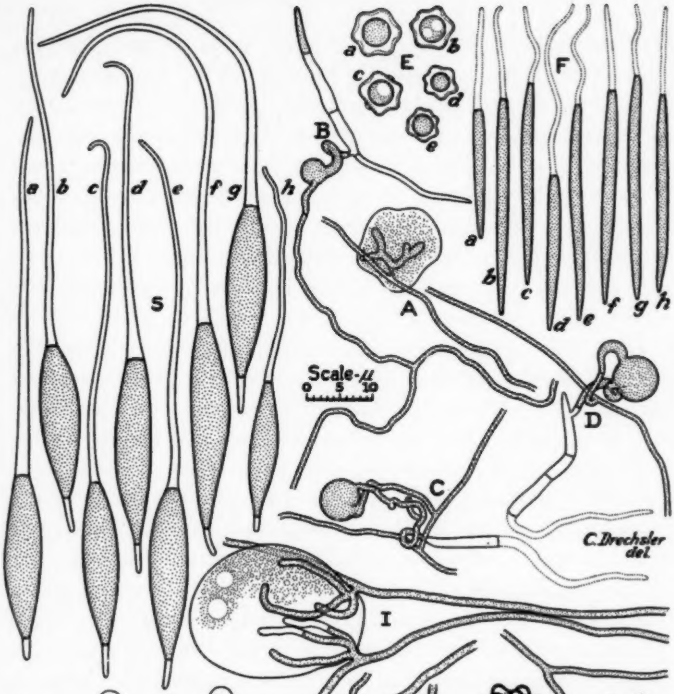

FIG. 3. A–F, *Acaulopage rhicnospora*. G–R, *Acaulopage ceratospora*.

pede physically the locomotion entailed in the feeding activities of *Amoebae* and nematodes. If fungi of such character are first to establish themselves in a Petri dish culture, prospects for the development of predacious forms like those herein discussed, are decidedly poor. On the other hand, if the culture is first occupied by various widely distributed species of *Aphanomyces* or *Pythium*, whose moist superficial mycelium is very favorable to bacterial development, and whose aerial growth soon collapses to furnish an unimpeded field for feeding, a number of predacious forms may appear in quantity.

The abundance of predacious fungi that appear in isolation plate cultures also depends greatly on the conditions attending incubation. The high temperatures prevailing during summer in Washington, D. C., are generally unfavorable, perhaps mainly because they inhibit the multiplication of infesting animals. Unless, therefore, refrigeration is resorted to neither *Amoeba*-capturing nor nema-capturing fungi are likely to be encountered often during the season in which the isolation especially of organisms causing plant diseases, is most actively carried on. Scarcely less important than temperature is the presence of moisture in available form. A hard culture medium as, for example, maizemeal agar containing over 30 grams of agar-agar to the liter, is often too firm in consistency to permit protozoans or nematodes to force their way through it; besides being sometimes so lacking in free superficial moisture that the animals may be impeded even in their movements on the surface, and perhaps also in a measure starved for want of an adequate supply of bacterial slime. Indeed, even when softer media containing 15 to 20 grams of agar-agar to the liter are employed in Petri dish cultures, *Amoebae* and nematodes and the fungi preying on them flourish much better if surface evaporation is reduced through confinement in a fairly tight container.

In view of the difficulties attending the adventitious development of predacious fungi the frequency with which they yet make their appearance in isolation plate cultures, is remarkable. Almost any bit of decaying vegetable matter that has been in contact with the moist ground for any protracted period of time, can with appropriate handling be made to yield several of them. There is good reason to believe that the fungi herein described as new, by no

means constitute mycological rarities, but deserve rather to be reckoned among the more nearly ubiquitous of plants. Moreover, as the eight species were encountered altogether incidently in the course of only a few months of observation directed primarily toward other objects, it may be presumed that a purposeful search would uncover a much larger number of related forms.

In method of holding their prey the eight species show much uniformity. An *Amoeba* after capture is always to be seen attached, whether to a mycelial element, or, as is often the case in some species, to a fallen conidium, by means of a minute mass of golden yellow adhesive material. From the mycelial element or the conidium is thrust forth a narrow process which passes through the deposit of adhesive material and perforates the animal's pellicle to give rise inside to a more or less characteristically branched haustorium or haustorial system. When the protoplasmic contents of the *Amoeba* are nearing exhaustion, the protoplasm of the haustorium begins to withdraw back into the parent mycelial filament. Eventually the haustorium is completely evacuated and thereupon, like the collapsed pellicle surrounding it, becomes altogether invisible; so that an instance of capture is afterwards found recorded, and then usually only rather dubiously, in a somewhat inconspicuous scar-like or slightly protuberant modification in the contour of the hypha or conidium.

As all attempts to isolate the predacious forms under discussion have failed, it is not known whether adhesive material would be elaborated by them in pure culture, removed from the presence of *Amoebae* as well as from any physical activity simulating the movements of these protozoans. In some of the larger forms the glutinous material may often be clearly seen as minute yellow lumps directly attached to the hyphae at irregular intervals; and even more minute lumps can sometimes be made out though necessarily with greater difficulty, in examining the hyphae and conidia of the more delicate species. On several occasions while observations were being made on the struggles of a captured *Amoeba* to free itself, it was noticed that stretches of the engaged filament in either direction from the prey, on which at first no yellow lumps had been evident, bore unmistakably a number of such lumps an hour or two later. A responsiveness to environmental conditions

is possibly involved here, comparable to that manifested by the predacious Hyphomycetes, all of which have so far consistently failed to produce organs of capture when grown undisturbed in pure culture.

Of living structural parts constituting in their connection with the yellow adhesive material rudimentary organs of capture, the Zygomycetes treated herein offer only a meager and somewhat questionable display. In the several species with sessile bush-like haustorial systems nothing suggestive of prehensile structures have been seen; nor would it seem readily possible that such structures could here be present. However, in species having stalked haustorial systems, short delicate processes with slightly expanded tips have been observed projecting from filaments (FIG. 2, E, F; FIG. 5, J) or from detached conidia (FIG. 5, J; **3**, *fig. 4, D*). These processes correspond closely to the lateral spurs on which newly captured *Amoebae* are held (FIG. 2, A, B, D, E; **3**, *fig. 4, B, C*); and would seem, therefore, to represent special prehensile structures, which after growing through the pellicles of the prey and branching dichotomously inside, come to make up the stalks of the haustorial systems. This interpretation is not necessarily contradicted by the fact that the haustorial stalk is often found wholly inserted in the captured animal (FIG. 2, C. J; **3**, *figs. 3, B, D*; *5, B*) since such positional relationship might as readily result from a captured animal engulfing a ready-formed prehensile process, as from the stalk growing into the animal after its capture. Unfortunately the adhesive material that might enable identification of the processes in question as prehensile structures beforehand is generally too minute in quantity to be discerned at all clearly. To add to the difficulties of interpretation, it happens that in species producing conidia directly on prostrate hyphae, the stumps (FIG. 2, B, *d*; H, *b*) left after disarticulation of these conidia have approximately the same dimensions as the processes presumed to function in the capture of prey (FIG. 2, E, *a–c*; F, *a–f*).

Though the apparatus of capture is in any case extremely simple, it is nevertheless decidedly efficacious in operation,—a circumstance to which the physical feebleness of *Amoebae* generally, combined with the relative firmness and durability of the pellicle recognizable more especially in the terricolous types of these animals, may

contribute in no small measure. Yet the efficacy of adhesive material, unaided by any sort of structural engagement, is revealed not only in *Amoeba*-capturing members of the Zoopagaceae but also in a nematode-capturing member of the family that was figured earlier (**2**, *fig. 8, A, C*) and is now more fully discussed elsewhere (**7**) under the binomial *Stylopage hadra*. Indeed, essentially the same method of capture is known among the predacious Hyphomycetes. For although the stalked, glandular knob-cells in the nema-capturing fungus illustrated synoptically (**2**, *fig. 7, A–C*) and subsequently (**4**) identified as *Dactylella ellipsospora* Grove (**8**) (=*Monosporidium repens* Zopf (**10**), =*Monacrosporium leporium* Bubák (**1**), =*M. elegans* Rostrup (**9**) non Oudemans), as well as the sessile elongate-ellipsoidal glandular cells produced by the rhizopod-capturing *Pedilospora dactylopaga* Drechsl. (**5**), are morphologically differentiated organs, they clearly operate altogether by adhesion.

SPECIES WITH SESSILE OF NEARLY SESSILE CONIDIA

In five of the species the conidia are borne directly on the hyphae creeping on the surface of the substratum, which make up a large part of the vegetative mycelium. Apparently any superficial hyphal element is capable of giving rise to asexual spores, proximity to the air and adequate nourishment constituting the only obvious requirements for such reproduction. Each conidium develops as an erect aerial outgrowth from the parent filament. After disarticulation a basal stump remains, which, although longer in some species than in others, is in none worthy of being considered a conidiophore. The lateral attachment of the conidia to the prostrate filaments provides a partial similarity to *Endocochlus asteroides* that is sustained in the presence of terminal appendages on the conidia of some of the forms. Yet in view of the pronounced dissimilarity in morphology of the vegetative thallus, reference to *Endocochlus* seems definitely precluded. A new genus is therefore proposed under a name intended to bring into relief the absence of distinct conidiophores.

Acaulopage gen. nov.

Mycelium effusum; hyphis continuis, hyalinis, parce ramosis, materia glutinosa flavida animalia minuta tenentibus, ramo pelliculam eorum pene-

trantibus, tum haustorium intus evolventibus et carnem vel protoplasma exhaurientibus. Conidia aerea, incolorata, hinc illinc ex hyphis repentibus assurgentia oriunda. Zygosporangia globosa in materia subjacenti e copulatione duarum similium hypharum orta.

Mycelium spreading; hyphae continuous, hyaline, rather sparingly branched, capturing minute animals by means of yellowish adhesive material, penetrating the pellicle or integument of each by means of a lateral branch, then producing a haustorium within that exhausts the fleshy or protoplasmic contents. Conidia aerial, colorless, arising erect at intervals from prostrate hyphae. Zygosporangia globose, produced in the substratum through the union of two similar filaments.

ACAULOPAGE RHAPHIDOSPORA

Of the forms eligible for inclusion in the genus perhaps the simplest one morphologically is to be recognized in the species (FIG. 1) having acicular conidia without appendages, that was figured earlier (**3**, *fig. 4, A–E*). This species has been seen rather frequently on old agar plate cultures, though it well deserves to be reckoned among the most inconspicuous of organisms. It can be detected most readily by very carefully examining with a dry objective of fairly high magnification the superficial growth present in old plate cultures, especially in areas immediately surrounding the pieces of decaying vegetable material used in starting them originally. The conidia (FIG. 1, E, *a–h*) in such an examination, are to be discerned as rather sparsely distributed needle-like structures, which, projecting from the surface of the substratum nearly vertically into the air, come into view, for the most part, only endways. For a more satisfactory inspection of the conidia, and for any view of the mycelium whatever, a thin surface layer of the substratum may be sliced off with a moistened razor, carefully removed to a slide, covered with a thin cover-glass, and examined under a water-immersion or oil-immersion objective of high magnification.

In a mount thus prepared the mycelium is seen to consist of filaments (FIG. 1, A–D, F) so delicate that they are exceeded in width by many of the bacteria among which they ramify. The short elements that make up the dichotomously branched haustorium visible within many of the captured *Amoebae* (FIG. 1,

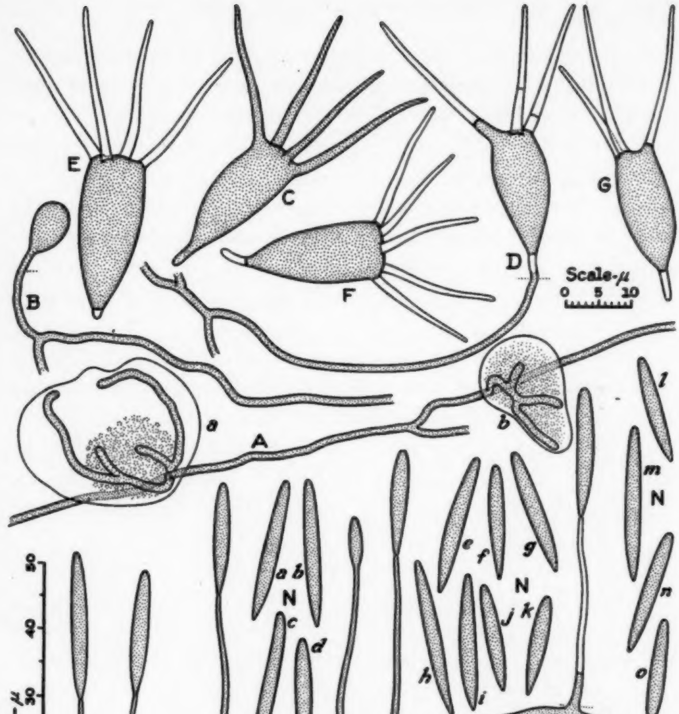

FIG. 4. A–G, *Acaulopage tetraceros*. H–N, *Stylopage haploe*.

A–D) are about twice as wide as the mycelial filaments generally. Haustoria within the larger animals are more extensive and more abundantly branched than those in the smaller ones. The *Amoebae* caught include mostly specimens ranging in diameter between 10 μ and 25 μ. In most of the larger newly captured specimens and also in some of smaller sizes, a subspherical nucleus can be made out (FIG. 1, A, C; **3**, *fig. 4, B*). This nucleus together with other less definite features suggests that the usual prey consists of the smaller and the medium-sized individuals of *Amoeba sphaeronucleolus* Greeff.

Sexual apparatus is formed both on and under the surface of the substratum. Two outwardly undifferentiated hyphal branches meet, fuse at their tips, and from the place of fusion give rise to the globose zygosporangium (FIG. 1, F). A septum makes its appearance in each of the fusing hyphae, though apparently not until the zygosporangium has attained nearly its definite size (FIG. 1, G, H). It appears probable that in spite of this tardiness the delimited portions of filament are approximately homologous to the gametangia of the more familiar Zygomycetes. In any case their contents pass into the zygosporangium in much the same way as the protoplasm of gametangia generally. The zygosporangium thereupon becomes walled off as a spherical cell, and develops internally a zygospore which at maturity has a relatively thick wall with bullate protuberances. Over this sculptured zygospore the slightly relaxed zygosporangial membrane collapses, sometimes rather closely, so that an arrangement of parts very similar to the arrangement in the sexual apparatus of *Zoopage phanera* is brought about (FIG. 1, I–L).

A specific term having reference to the needle-like shape of the conidia seems appropriate for the fungus.

Acaulopage rhaphidospora sp. nov.

Sparsa; hyphis incoloratis, .6–.9 μ crassis, haustoria dichotoma ex ramulis 1–1.5 μ crassis composita evolventibus. Conidia continua, paulo acicularia, recta vel leniter curvata, 25–45 μ, saepius 30–40 μ, longa, 1.2–1.7 μ crassa. Zygosporangia primo levia, sphaeroidea, 5–7 μ diam., in maturitate membrana circa zygosporam collabente; zygospora incolorata vel flavida, sphaeroidea, 4.5–6.5 μ diam., membrana .5–1.3 μ crassa, 10–25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* minores, saepius 10–25 μ latas, quae magnam partem probabiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .6 to .9 μ wide, producing haustoria consisting of branches 1 to 1.5 μ wide. Conidia continuous, somewhat needle-shaped, straight or slightly curved, 25 to 45 μ, mostly 30 to 40 μ long, and 1.2 to 1.7 μ wide. Zygosporangium at first smooth, spherical, 5 to 7 μ in diameter, its wall at maturity collapsing rather closely around the zygospore; the zygospore colorless or yellowish, subspherical, 4.5–6.5 μ in diameter, with a wall .5 to 1.3 μ thick and ornamented with 10–25 bullate protuberances.

Occurring in soil and decaying plant materials, capturing and consuming smaller *Amoebae* that measure often 10 to 25 μ in diameter and belong probably in large part to *Amoeba sphaeronucleolus;* near Washington, D. C.

ACAULOPAGE MACROSPORA

A species very similar to the one just described but having generally greater dimensions was found in some quantity in a single agar plate culture. Its mycelial filaments (FIG. 2, A–H), though far from coarse, are approximately twice as wide as those of *Acaulopage rhaphidospora.* The *Amoebae* that are captured on them and on the conidia include animals larger than any taken by *A. rhaphidospora,* as well as extremely minute individuals (FIG. 2, A–E, J). They reveal, however, essentially the same morphological features as those preyed upon by *A. rhaphidospora,* and would seem likewise referable, in the main, to *Amoeba sphaeronucleolus.* Correlated evidently with the generally greater size of the animals captured is a more extensive development of the haustorial system (FIG. 1, A). The haustorial branches are of about the same width as the mycelial hyphae from which they have origin, thus providing a contrast in dimensional relationships with the species already described, wherein the homologous elements are conspicuously wider than the ordinary filaments.

Sexual apparatus was found associated with the fungus, though only in small quantity and in apparently immature condition (FIG. 2, K). As in other members of the group paired branches regularly arise from separate hyphae. The zygosporangium, while half again as large in diameter as that of *Acaulopage rhaphidospora,* is similarly smoothly spherical on attaining full size, and likewise shrinks somewhat with the contraction of protoplasmic contents incident to the development of the zygospore proper.

Conidia (FIG. 2, I, *a–z*) were produced by the fungus in greater quantity than in any non-catenulate member of the Zoopagaceae observed so far. Examined under a dry objective they stood forth from the substratum in a conspicuously bristling array. In length and in width they exceed the conidia of *Acaulospora rhaphidospora* by approximately a half, and taper less markedly toward apex and base, which as a consequence are more bluntly rounded off. In some conidia the contents were found withdrawn from one or the other of the ends, leaving the empty portion attached as an appendage (FIG. 2, I, *d, f, t, x, z*) comparable, no doubt, with the more distinctive conidial appendages present in other members of the group. Apical bifurcation was seen in some conidia, occurring (FIG. 2, I, *k, r*) apparently as an occasional irregularity.

The sterigmata on which the conidia are borne (FIG. 2, G; H, *a*) represent in this species structures more substantial than in any of the other forms placed in the genus, remaining behind after disarticulation of the spores as tapering projections about 3 μ long (FIG. 2, B, *d*; H, *b*). Lateral processes rather similar to them except in being bent or contorted in various ways, were observed on some of the hyphae (FIG. 2, E, *a–c*; F, *a–f*). The difficulty of interpreting these processes has already been referred to. It is not impossible that sterigmata might be constrained into conspicuous irregularity of form through changes in position of the parent hyphae such as might be effected, perchance, by the jostling of young earthworms or of the larger nematodes. Somewhat more plausibility, however, would seem to attach to the explanation that the processes constitute prehensile contrivances, which, after successfully intercepting and holding prey, penetrate into the animals to form the stalked haustorial systems characteristic of the species.

A term having reference to the unusual length of the conidia is deemed appropriate as a specific name for the fungus.

Acaulopage macrospora sp. nov.

Paulo sparsa; hyphis incoloratis 1–2 μ crassis, haustoria divaricata usque ter vel quater repetite irregulariter bifurcata evolventibus. Conidia elongato-cylindracea, utrimque leniter attenuata et abrupte rotundata, 30–70 μ longa, 1.6–2.5 μ crassa, sed interdum sursum bifurcata, etiam interdum parte infera vel parte supera evacuata. Zygosporangia primo levia, sphaeroidea, circiter 9 μ diam., membrana ad maturitatem circa zygosporam leniter collabente.

Habitat in radicibus putrescentibus, *Amoebas* 5–40 μ latas, quae magnam partem probabiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Somewhat sparse; hyphae colorless, 1–2 μ wide, giving rise to spreading haustoria irregularly dichotomously branched up to 3 or 4 times. Conidia elongate-cylindrical, tapering gradually toward the abruptly rounded basal and distal ends, 30 to 70 μ long, 1.6 to 2.5 μ wide, but sometimes distally bifurcate, and sometimes also, with basal or distal portion evacuated. Zygosporangium at first smooth, subspherical, approximately 9 μ in diameter, with a wall collapsing somewhat about the zygospore towards maturity.

Occurring in decaying roots, capturing and consuming *Amoebae* 5 to 40 μ in diameter, probably belonging mostly to *Amoeba sphaeronucleolus,* near Washington, D. C.

ACAULOPAGE RHICNOSPORA

The tendency toward evacuation of a portion of the conidium expressed occasionally in *Acaulopage macrospora,* is manifested with much regularity in a species otherwise closely resembling *A. rhaphidospora.* When undisturbed material of this species in a Petri dish culture is examined under a dry objective, the conidia directed nearly vertically are seen to terminate individually in a shriveled collapsed prolongation usually somewhat shorter than the basal part (FIG. 3, F, *a–c, f–h*; 3, *fig. 5, C, b*), but sometimes equally long or even slightly longer (FIG. 3, F, *d*). When such material is mounted in water, the empty appendage becomes all but invisible even under the best of immersion objectives, so that its position and size are often revealed only through its interruption of the rather uniformly granular field that the development of bacteria on the surface of the substratum ordinarily provides.

Although the shriveled appendages make for a distinctive appearance, their presence can hardly be considered an altogether decisive diagnostic character. The possibility that the very slender conidia bearing them may represent merely conidia of *Acaulopage rhaphidospora* that have become evacuated in the distal portions, perhaps through development coming with increasing age, is difficult to dispose of conclusively. However, observations repeated at intervals on stands of acicular conidia devoid of appendages did not disclose apical evacuation on any considerable scale;

whereas in stands displaying appendages apparently in conformity with a usual structural peculiarity, the necessary apical evacuation seemed to have occurred soon after the conidia attained their definitive dimensions. Since, moreover, the appendaged conidia in general slightly exceeded the acicular ones both in total length and in width, it would seem somewhat safer to consider them as being produced by a separate species.

The mycelium like that of *Acaulopage rhaphidospora* is composed of extraordinarily delicate filaments (FIG. 3, A–D; **3**, *fig. 5, B*). Within the minute *Amoebae* caught on these filaments are produced haustoria, which consist, as in *A. rhaphidospora*, of a few thicker but relatively short branches borne dichotomously on a short delicate stalk (FIG. 3, A; **3**, *fig. 5, B*). Sexual apparatus is formed readily and in moderate abundance. The fusing filaments arise consistently from separate elements. Sometimes two branches from separate mycelial filaments are represented in the union (**3**, *fig. 5, E, a*), sometimes two germ tubes from separate conidia (**3**, *fig. 5, E, b*), and sometimes a hyphal branch paired with a germ tube from a conidium (FIG. 3, B–D). The wall of the originally smooth subspherical zygosporangium appears at maturity to collapse closely about the sculptured zygospore proper (FIG. 3, E, *a–e*); so that in relationship of parts as well as in development, the sexual apparatus offers a rather accurate parallelism with that of *Zoopage phanera*.

A term having reference to the withered aspect of the conidia would seem appropriate as a specific name for the fungus.

Acaulopage rhicnospora sp. nov.

Sparsa; hyphis incoloratis, .6–.9 μ crassis, haustoria dichotoma ex ramulis 1–1.5 μ crassis composita evolventibus. Conidia hyalina, 20–55 μ longa, 1.5–2 μ crassa, parte supera saepius in maturitate evacuata itaque appendicula marcida constituens, parte infera deorsum attenuata. Zygosporangia primo levia, sphaeroidea, 4.5–7 μ diam., in maturitate membrana circa zygosporam collabente; zygospora incolorata vel flavida, sphaeroidea, 4–6.5 μ diam., membrana .5–1.3 μ crassa, 10–25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* minores saepius 10–15 μ latas capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .6 to .9 μ wide, producing dichotomously branched haustoria with branches 1 to 1.5 μ wide. Conidium hyaline, 20 to 55 μ long, 1.5 to 2 μ wide, the distal part at

maturity often evacuated and then constituting a withered appendage, the proximal part tapering toward the base. Zygosporangium at first smooth, subspherical, 4.5 to 7 μ in diameter, its wall at maturity collapsing rather closely about the zygospore; the zygospore colorless or yellowish, subspherical, 4 to 6.5 μ in diameter, with a wall .5 to 1.3 μ thick and ornamented with 10 to 25 bullate protuberances.

Occurring in soil and in decaying plant materials, capturing and consuming the smaller *Amoebae* that measure mostly 10–15 μ in diameter, near Washington, D. C.

ACAULOPAGE CERATOSPORA

Of all fungi predacious on *Amoebae* the one that has been observed most frequently is a species of *Acaulopage* having a mycelium only slightly more delicate than that of *A. macrospora*. The *Amoebae* caught on the mycelial filaments (FIG. 3, G–I) include small and medium-sized animals, at least some of which appear to correspond fairly satisfactorily in morphology to *Amoeba sphaeronucleolus*. The haustorium shows a basal, bush-like type of branching rather different from the dichotomous branching characteristic of the three congeneric forms already described. The branches, moreover, are approximately of the same diameter as the mycelial filament from which they arise, providing therefore a contrast to the relationship evident in *A. rhaphidospora* and *A. rhicnospora* where the haustorial branches are conspicuously wider than the hyphae generally.

Though produced rather sparingly even on well-nourished mycelia, the conidia (FIG. 3, S, *a–h*) arrest attention both by their dimensions and their distinctive structure. On full maturity, the finely granular protoplasm of the asexual spore is concentrated in an elongated ellipsoidal cell. At its narrow proximal end this cell is delimited by a small septum from a short, narrow, empty basal appendage; at its more broadly truncated distal end it is delimited by a larger septum from a long, empty, tapering appendage. The distal appendage, usually half again or twice as long as the living cell, appears, like that of *Acaulopage rhicnospora*, shriveled when viewed in its natural state on the dry substratum. The evacuated membrane composing it, however, is thick enough in the present

species that it can be made out clearly in a moist preparation under a good immersion objective.

Sexual apparatus is sometimes formed in moderate quantity, but more often is completely absent. It has not been possible to determine definitely whether the fungus is heterothallic; though, as in other members of the genus, the two conjugating branches always arise from separate filaments (FIG. 3, J–L). The fully grown zygosporangium, unlike the homologous structure in *Acaulopage rhaphidospora* and *A. rhicnospora,* is moderately sturdy and rather beautifully ornamented with bullate protuberances (FIG. 3, M–R). As far as can be determined under the optical difficulties introduced by the sculpturing of the zygosporangium, the zygospore proper is surrounded by a smooth spherical wall. Thus, whereas both of the sexual hyphae are connected directly with the zygosporangium, as in *Zoopage phanera,* the sculpturing and eventual shape of mature zygosporangium and zygospore show parallelism rather with *Bdellospora helicoides.*

A term having reference to the hornlike shape of the distal conidial appendage is deemed appropriate as a specific name for the fungus.

Acaulopage ceratospora sp. nov.

Sparsa; hyphis incoloratis, .9–1.8 μ crassis, haustoria arbusculiformia divaricata ex aliquot ramulis composita evolventibus. Conidia hyalina, in totam 60–110 μ longa, tripertita: parte supera vacua, sursum attenuata, paulo subulata, 30–70 μ longa, basi 1–3 μ crassa, apice .5–.8 crassa, appendicula saepe marcida facta; parte media protoplasmatis viventis repleta, elongato-ellipsoidea, 20–34 μ longa, 4–6 μ lata; parte infima vacua, saepe deorsum paulo attenuata, 2–6 μ longa, .8–1.2 μ crassa. Zygosporangia flavida, sphaeroidea, 6–11 μ diam., 15–25 verrucis ornata; verrucis .5–1.5 μ altis, 1.5–3 μ diam. Zygosporae globosae, verisimiliter leves, membrana crassa, loculo interno 4.5–8 μ diam.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* quae parte probabiliter *Amoebae sphaeronucleoli* sunt capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .9 to 1.8 μ wide, producing bushlike spreading haustoria consisting of several branches. Conidium hyaline, 60 to 110 μ in total length, consisting of three parts: a distal, tapering, somewhat awl-shaped empty part, present under dry conditions as a withered appendage, 30–70 μ long, 1 to 3 μ wide at its base and .5 to .8 μ wide at its tip; a middle part filled with living protoplasm, elongate ellipsoidal, 20 to 34 μ long, 4 to 6 μ

wide; a lower empty part often tapering somewhat toward the base, 2 to 6 μ long, .8 to 1.2 μ wide. Zygosporangium yellowish, subspherical, 6 to 11 μ in diameter, ornamented with 15 to 25 warty protuberances, which are .5 to 1.5 μ high and 1.5 to 3 μ in basal diameter. Zygospore globose, apparently smooth, and a thick membrane surrounding a loculus 4.5 to 8 μ in diameter.

Occurring in soil and in decaying plant materials, capturing and consuming *Amoebae* in part belonging probably to *Amoeba sphaeronucleolus,* near Washington, D. C.

ACAULOPAGE TETRACEROS

An even more conspicuous development of empty conidial appendages than is found in *Acaulopage ceratospora* provides the chief distinctive feature of a fungus often encountered on old isolation plate cultures, and on pieces of decaying plant materials that have been kept partly bathed in water for some days. In either cultural environment the fungus apparently subsists entirely on *Amoebae,* the animals captured by it being mostly of the smaller sizes. The haustorial system within the prey, is disposed in a bushlike manner somewhat like the haustorial system of *A. ceratospora,* which it resembles besides in that the elements composing it are approximately of the same diameter as the parent mycelial filament (FIG. 4, A, *a, b*; **3**, *fig. 2, B*).

The conidia, which for the most part are produced rather sparingly, consist individually of a large inversely flask-shaped cell together with a short basal stipe and from 2 to 6, mostly 3 to 5, gradually tapering empty distal appendages. In the earlier stages of its development the conidium first appears as a terminal bulbous enlargement on a very short erect branch arising from a prostrate filament, or on a short erect terminal prolongation of such a filament (FIG. 4, B). From the distal end of the growing enlargement are then thrust forth, in spreading, approximately symmetrical arrangement, the several branches (FIG. 4, C; **3**, *fig. 2, A*) that later through the withdrawal of the protoplasm are converted into the empty subulate appendages. Sometimes apparently this withdrawal is interrupted for a period long enough to entail the laying down of a median partition (FIG. 4, D). On maturity disarticulation occurs a short distance below the point where the

filament widens out. As the short cylindrical part thereby included in the conidium, and comparable to the neck of the inverted flask corresponding to the living cell, has generally been evacuated before disarticulation takes place, it usually presents itself subsequently as the empty basal stipe (FIG. 4, E–G; **3**, *fig. 2, C*) already mentioned.

The conidium thus constituted has an appearance little suggestive of phycomycetous affinities, being reminiscent, even if somewhat vaguely, rather of genera in the Mucedinaceae-Staurosporae. That at least two fungi eligible for inclusion in the latter group—the quadrilobate *Monacrosporium*-like form figured earlier (**2**, *fig. 9, A, C*), and *Pedilospora dactylopaga*—subsist through the capture of microscopic invertebrates, contributes to a remarkable parallelism. It is difficult to avoid the presumption that in some manner the curious modifications in the conidia of these Hyphomycetes, and also the similar modifications in the conidia of the phycomycete under discussion, must be related to the predacious habit that these fungi have in common. In cultures of irrigated vegetable materials, as was pointed out previously, conidia of the present fungus keep afloat on the surface of the water, mainly, no doubt, owing to the buoyancy of the empty appendages. The evident utility of the appendages as flotative devices need, however, not preclude other and perhaps more essential usefulness.

The evident relationship of the fungus especially to *Acaulopage ceratospora* would seem to justify, for the time being at least, assignment to the same genus. A term having reference to four hornlike appendages—four being approximately the average number found, as well as the number most often actually present—is deemed sufficiently accurate in arithmetical connotation to be suitable as specific name.

Acaulopage tetraceros sp. nov.

Sparsa; hyphis incoloratis, .9–1.8 μ crassis, haustoria arbuculiformia interdum parte dichotoma evolventibus. Conidia hyalina basi stipitata, apice 2–6 (saepe 3–5) appendicibus divergentibus vestita: cellula viventi protoplasmatis repleta, inversum lageniformis, 16–24 μ (saepius circiter 20 μ) longa, 7–10 μ (saepe circa 8 μ) lata; stipite vacuo, 1–5 μ longo, .8–1.5 μ lato; appendicibus circum apicem latum cellulae viventis dispositis, vacuis, subulatis, 14–26 μ (saepe circa 20 μ) longis, basi 1–2 μ crassis. Zygosporae ignotae.

Habitat in terra et in materiis diversis plantarum putrescentibus, *Amoebas* minores quae parte probabiliter *Amoebae sphaeronucleoli* sunt capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .9 to 1.8 μ wide, producing bushlike haustoria that sometimes are in part dichotomously branched. Conidium hyaline, stipitate at the base, furnished at the apex with 2 to 6, mostly 3 to 5, divergent appendages: the living cell filled with protoplasm, inversely flask-shaped, 16 to 24 μ (mostly about 20 μ) long, and 7 to 10 μ (mostly about 8 μ) wide; the stipe empty, 1 to 5 μ long, and .8–1.5 μ wide; the appendages arranged rather symmetrically about the broad distal end of the living cell, devoid of protoplasmic contents, awl-shaped, 14 to 26 μ (mostly about 20 μ) long, and individually 1 to 2 μ wide at the base. Zygospores unknown.

Occurring in the soil and in different decaying plant materials, capturing and consuming smaller *Amoebae* that probably belong in part to *Amoeba sphaeronucleolus,* near Washington, D. C.

SPECIES WITH CONIDIA BORNE ON ERECT CONIDIOPHORES

In a number of species closely similar to those described under the genus *Acaulopage* the conidia are borne on erect hyphae, which though not differing much from the vegetative filaments in structural details, are functionally quite distinct in being given up exclusively to asexual reproduction. As has been noted previously these species show nothing of the tendency toward the development of empty conidial appendages evident in *Acaulopage.* It may be inferred with some little plausibility perhaps that in elevating the spore to a position well above the substratum, the ecological need for appendages is obviated. However, even if a divergence in ecological relationship were not to be assumed, the divergence in morphology would yet seem so decisive as to dictate a corresponding taxonomic separation. A separate genus is therefore proposed, under a name intended to bring into relief the presence of conidiophores as well as to make reference to the predacious character that the fungi in question share with members of other genera.

Stylopage gen. nov.

Mycelium effusum; hyphis sterilibus continuis, hyalinis, parce ramosis, materia glutinosa flavida animalia minuta tenentibus, ramo pelliculam eorum

penetrantibus, tum haustorium intus evolventibus et carnem vel protoplasma exhaurientibus; hyphis fertilibus erectis, unicum conidium apice ferentibus vel plura conidia singulatim post incrementa repetita ferentibus. Conidia hyalina, incolorata. Zygosporangia globosa, intra materiam subjacentem e copulatione duarum similium hypharum orta.

Mycelium effuse; vegetative hyphae continuous, hyaline, rather sparingly branching, holding minute animals by means of yellowish adhesive material, penetrating the pellicle or integument of each by means of a lateral branch, then producing a haustorium, or an internal mycelium, which exhausts the fleshy or protoplasmic contents; fertile hypha erect, bearing a single conidium at its apex, or, following repeated elongation, several conidia produced successively. Conidia hyaline, colorless. Zygosporangium globose, produced in the substratum from the union of two similar hyphae.

STYLOPAGE HAPLOE

Of the several known species referable to the genus the one having at once the shortest and simplest conidiophores, and therefore showing the smallest departure in morphology from *Acaulopage,* appears to be relatively scarce, having been encountered only twice on old isolation agar plate cultures. Its vegetative mycelium (FIG. 4, H–M), if slightly more delicate than that of *A. macrospora,* captures *Amoebae* that not only are of approximately the same range of dimensions (FIG. 4, H–J) but also appear assignable in large part to *Amoeba sphaeronucleolus.* The parallelism is extended in the dichotomous branching and limited spread of the haustorial system developed within the prey (FIG. 4, H–J). The conidia (FIG. 4, N, *a–o*), however, are much smaller than those of *A. macrospora.* They resemble rather closely those of the much more delicate form described herein as *Stylopage araea,* though their somewhat greater dimensions and generally more bluntly rounded apical ends become sufficiently evident as distinguishing features on more careful comparison. Production of successive conidia following repeated elongation of the conidiophore has never been observed in this species; though it might be unsafe to assume that such reproductive development could not occur, for example, in especially well nourished material. A term having reference to the simplicity of the conidial apparatus (FIG. 4, I, *a, b*; K, *a, b*; L; M) is deemed reasonably appropriate as a specific name for the fungus.

Stylopage haploe sp. nov.

Sparsa; hyphis sterilibus incoloratis, 1–1.7 μ crassis, haustoria irregulariter dichotoma divaricata evolventibus; hyphis fertilibus incoloratis, 25–40 μ altis, basi saepius 1–1.2 μ crassis, sursum paulatim attenuatis, apice .5–.8 μ crassis, unicum conidium terminale ferentibus. Conidia paulo fusoidea, basi acuta, apice plus minusve rotundata, 15–25 μ (saepius circiter 19 μ) longa, 2.2–2.7 μ (saepius circiter 2.4 μ) crassa. Zygosporae ignotae.

Habitat in materiis plantarum putrescentibus, *Amoebas* saepius usque 40 μ diam., quae magnam partem probabiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; sterile hyphae colorless, 1–1.7 μ wide, producing irregularly dichotomous spreading haustoria; fertile hypha colorless, 25 to 40 μ high, 1 to 1.2 μ wide at the base, tapering upward gradually, .5 to .8 μ at the tip, bearing a single terminal conidium. Conidium somewhat fusoid, rather acutely pointed at the proximal end, thicker and more or less bluntly rounded at the distal end, 15 to 25 μ (mostly about 19 μ) long, 2.2–2.7 μ (mostly about 2.4 μ) wide. Zygospores not known.

Occurring in decaying plant materials, capturing and consuming *Amoebae* up to 40 μ in diameter, probably belonging in large part to *Amoeba sphaeronucleolus,* near Washington, D. C.

STYLOPAGE ARAEA

A fungus showing the same general arrangement of parts as the one just described, but presenting a far different and much more graceful appearance, was observed rather frequently on old isolation plate cultures. Small *Amoebae* as well as medium-sized *Amoebae* measuring up to 50 μ in diameter and mostly referable apparently to *Amoeba sphaeronucleolus* are captured on its delicate mycelium (FIG. 5, A). The rangy bushlike haustorial system shows close basal branching, supplemented especially in instances of more extensive development in the larger animals with looser branching some distance above the base (FIG. 5, B, C). Haustorial elements and mycelial filaments are approximately equal in width. Undoubtedly the most distinctive feature of the fungus is found in the height and remarkable slenderness of the conidiophore (FIG. 4, D), which at first sight would appear hardly capable of supporting the sizable obovoid conidium (FIG. 5, E, *a–z, aa*) produced, as far as can be determined, always singly at its tip. A term meaning " slender " is accordingly proposed as appropriate for the species.

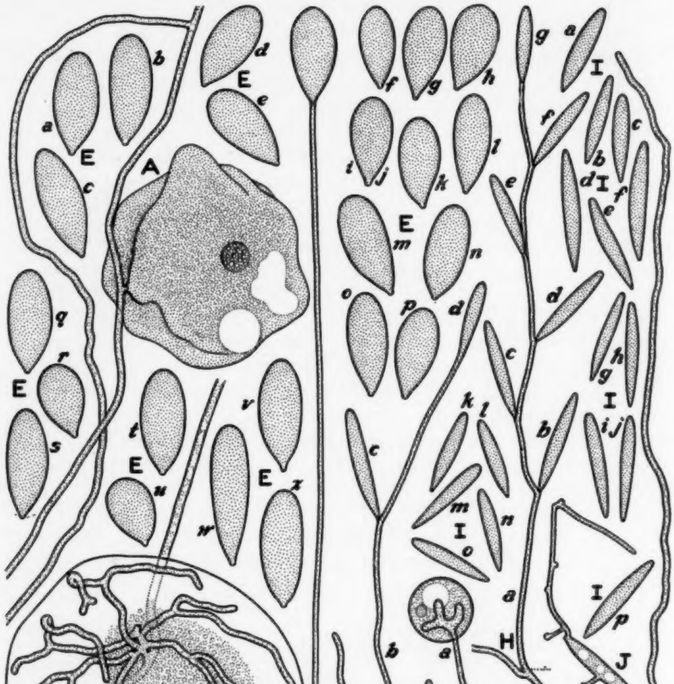

FIG. 5. A–F, *Stylopage araea.* G–M, *Stylopage lepte.*

Stylopage araea sp. nov.

Sparsa; hyphis sterilibus incoloratis, .8–1.3 μ crassis, haustoria divaricata arbusculiformia evolventibus; hyphis fertilibus incoloratis, 150–225 μ altis, .8–1 μ crassis, unicum conidium terminale ferentibus. Conidia incolorata, elongato-obovoidea, basi paulo apiculata, 10–22 μ (saepius circiter 15 μ) longa, 5.4–7 μ (saepius circiter 6.4 μ) lata. Zygosporae ignotae.

Habitat in materiis plantarum putrescentibus, *Amoebas* usque 50 μ diam., quarum multae verisimiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, .8 to 1.3 μ wide, producing spreading bushlike haustoria; fertile hypha colorless, 150 to 225 μ high, .8–1 μ wide, bearing a single terminal conidium. Conidium colorless, elongate-obovoid, somewhat apiculate at the base, 10 to 22 μ (mostly about 15 μ) long, 5.4 to 7 μ (mostly about 6.4 μ) wide. Zygospores not known.

Occurring in decaying plant materials, capturing and consuming *Amoebae* measuring up to 50 μ in diameter, the larger ones apparently belonging to *Amoeba sphaeronucleolus,* near Washington, D. C.

STYLOPAGE LEPTE

Making its appearance in isolation plate cultures more frequently than either of the two species of *Stylopage* already discussed, is a third form of which figures were included among the synoptic illustrations published earlier (**3**, *fig. 3*). As was indicated then the fungus in its vegetative stage closely resembles the two species described herein as *Acaulopage rhaphidospora* and *A. rhicnospora;* its extraordinarily narrow mycelial threads similarly capturing *Amoebae* of the smallest sizes and producing within each a dichotomously branching haustorium consisting of short widened elements (FIG. 5, G, *a*; **3**, *fig. 3, B*). Equally close similarity to the two species of *Acaulopage* is evident also in the sexual apparatus, the membrane of the originally smoothly spherical zygosporangium (FIG. 5, K, *a, b*; **3**, *fig. 3, E*) here likewise collapsing rather closely about the very small sculptured zygospore (FIG. 5, M), and thereby bringing about a relationship of parts much like that described earlier in the account of *Zoopage phanera.* In its asexual reproduction, however, the fungus is moderately distinctive. The erect conidiophores (FIG. 5, G, H; **3**, *fig. 3, A*), in spite of their frail appearance do not stop in their development after producing a single terminal conidium, but through repeated elongation very

often come to bear successively up to a half dozen conidia (FIG. 5, H, *b–g*) in the arrangement familiar, for example, in *Phytophthora infestans* (Mont.) De Bary. The conidia themselves (FIG. 5, I, *a–s*) have an obvious resemblance to those of the generally more robust *S. haploe,* but are somewhat smaller and because of their more marked apical tapering have, on the whole, a more distinctly fusoid shape. Besides giving rise to germ-tubes that grow out into delicate mycelia (FIG. 5, J), they often directly produce haustoria (**3**, *fig. 3, D*) within *Amoebae* that happen to come in contact with them.

A term having reference more especially to the frailness of the conidiophore would seem appropriate as specific name for this minute inconspicuous fungus.

Stylopage lepte sp. nov.

Sparsa; hyphis sterilibus incoloratis, .6–1 μ crassis, haustoria dichotoma ex ramulis 1–1.5 crassis composita evolventibus; hyphis fertilibus incoloratis, 25–100 μ altis, .7–.9 μ crassis, usque 6 conidia singulatim post incrementa repetita ferentibus. Conidia fusoidea, basi acuta, ad apicem rotundatum plus minusve attenuata, 12–19 μ (saepius circiter 15 μ) longa, 1.9–2.7 (saepius circiter 2.2 μ) crassa. Zygosporangia primo levia sphaeroidea, 5–7 μ diam. in maturitate membrana circa zygosporam collabenta; zygospora incolorata vel flavida, sphaeroidea, 4.5–6.5 μ diam., membrana .5–1.3 μ crassa, 10–25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* magnam partem 10–20 μ latas capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, .6 to 1 μ wide, producing haustoria consisting of branches 1–1.5 μ wide; fertile hyphae colorless, 25 to 100 μ high, .7 to .9 μ wide, bearing up to 6 conidia in succession after repeated elongation. Conidium fusoid, acute at the base, tapering more or less toward the sharply rounded apex, 12 to 19 μ (mostly about 15 μ) long, 1.9 to 2.7 (mostly about 2.2 μ) wide. Zygosporangium at first smooth subspherical, 5 to 7 μ in diameter, its wall at maturity collapsing about the zygospore; zygospore colorless or yellowish, subspherical, 4.5 to 6.5 μ in diameter, with a wall .5 to 1.3 μ thick and ornamented with 10 to 25 wartlike protuberances.

Occurring in soil and in decaying plant materials, capturing and consuming *Amoebae* mostly 10 to 20 μ in diameter, near Washington, D. C.

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EXPLANATION OF FIGURES

Fig. 1. *Acaulopage rhaphidospora;* drawn with the aid of the camera lucida at a uniform magnification; × 1000 throughout. *A–C,* Portions of hyphae with captured *Amoebae,* showing variations in the development of the haustorial system. *D,* Portion of hypha with two captured *Amoebae, a* and *b*. *E,* Conidia, *a–h,* showing variations in size and shape. *F,* Zygosporangium, nearly fully grown, at a stage preceding the appearance of septa in the conjugating branches. *G,* Young zygosporangium after appearance of a septum in one of the conjugating branches. *H,* Young zygosporangium after appearance of a septum in both of the conjugating branches. *I, J,* Mature sexual apparatus with hyphal connections, the dotted contour within each representing the optically uncertain outer profile of the zygospore wall. *K, L,* Mature zygosporangia with zygospores, as they appear after their mycelial connections are no longer visible.

Fig. 2. *Acaulopage macrospora;* drawn with the aid of the camera lucida at a uniform magnification; × 1000 throughout. *A,* Portion of branching hypha on which have been captured four *Amoebae, a–d;* showing variations in the dimensions of the animals, and corresponding differences in development of haustoria. *B,* Portion of branching hypha with three captured *Amoebae, a–c,* and showing a sterigma, *d*. *C, D,* Portions of hyphae, each with a captured *Amoeba*. *E,* Portion of hypha showing a captured *Amoeba* and three lateral processes, *a–c,* probably representing adhesive organs of capture. *F,* Portion of hypha with six lateral processes, *a–f*. *G,* Portion of prostrate hypha with a growing conidium; the surface of the substratum being indicated approximately in the dotted line. *H,* Hypha with one sterigma, *a,* bearing a fully developed conidium, and another sterigma, *b,* after

removal of conidium. *I*, Conidia, *a–z*, showing variations in size and shape, evacuation of apical (*d, f*) and proximal (*t, x, z*) portions, and distal bifurcation (*k, r*). *J*, Conidium with two minute *Amoebae*, *a* and *b*, captured by it. *K*, Sexual apparatus, showing diclinous origin of two zygosporangia, *a* and *b*.

Fig. 3. Drawn with the aid of the camera lucida at a uniform magnification; × 1000 throughout.

A–F, Acaulopage rhicnospora: A, Portion of hypha with a captured *Amoeba*. *B–D*, Immature sexual apparatus, each zygosporangium being formed from union of a mycelial branch and a germ tube produced by a conidium. *E*, Mature zygosporangia, *a–e*; the dotted contour in each indicating the optically obscure outer profile of zygospore wall. *F*, Conidia, *a–h*, showing variations in size, shape, and development of apical appendage.

G–R, Acaulopage ceratospora: G, H, Portions of hypha, each with a captured *Amoeba*. *I*, An *Amoeba* captured and being consumed by two separate hyphae. *J. K*, Sexual apparatus in early stage of development. *L*, Zygosporangium fully grown. *M–P*, Mature sexual apparatus with supporting branches. *Q*, Mature sexual apparatus: *a*, complete, in optical section; *b*, upper aspect of zygosporangial wall alone. *R*, Mature zygosporangium of nearly minimum size. In *M–R* the dotted contour represents the optically uncertain outer profile of the zygospore wall.

Fig. 4. Drawn with the aid of the camera lucida at a uniform magnification; × 1000 throughout.

A–G, Acaulopage tetraceros: A, Portion of hypha with two captured *Amoebae*. *B*, Portion of hypha with conidium in early stage of development, the dotted line indicating approximately the surface of the substratum. *C*, Conidium fully grown but immature, the stipe and appendages still being filled with protoplasm. *D*, Portion of hypha bearing a nearly mature conidium; the septum in each of the two completely evacuated appendages mark an interruption in the process of evacuation at approximately the same stage as that represented in the third appendage. *E–G*, Mature conidia showing variations in size and shape.

H–N, Stylopage haploe: H, Portion of hypha with a rather large captured *Amoeba*. *I*, Portion of hypha with a captured *Amoeba* and two erect conidiophores, *a* and *b*, each bearing a conidium. *J*, Conidium that in germinating gave rise to two germ tubes, in addition to producing a haustorial system within an *Amoeba* captured by it. *K*, Portion of hypha with two conidiophores, one, *a*, bearing a mature conidium, the other, *b*, a young conidium. *L*, Portion of hypha with conidiophore and mature conidium. *M*, Portion of hypha with largely evacuated conidiophore bearing a mature conidium. *N*, Conidia, *a–o*, showing variations in size and shape. The dotted lines in *I, K, L* and *M* indicate the approximate position of the surface of the substratum in relation to the individual conidiophores.

Fig. 5. Drawn with the aid of the camera lucida at a uniform magnification; × 1000 throughout.

A–F, Stylopage araea: A, Portion of hypha with newly captured *Amoeba*. *B*, Portion of hypha with a well developed haustorial system in the overlying captured *Amoeba*. *C*, Portion of hypha and the haustorial system in a badly depleted *Amoeba*, the attachment being shown in profile. *D*, Conidiophore

arising from a prostrate filament, and bearing a single terminal conidium; the surface of the substratum being indicated approximately by the dotted line. *E*, Conidia, *a–z*, *aa*, showing variations in size and shape. *F*, Germinating conidium still retaining half of its protoplasmic contents after having given rise to two germ tubes of considerable length.

G–M, *Stylopage lepte*: *G*, Portion of mycelium from which have been produced a haustorium (*a*) within a captured *Amoeba*, and a conidiophore (*b*) bearing a mature (*c*) and a young (*d*) conidium. *H*, Portion of hypha bearing a well developed conidiophore, *a*, with six conidia, *b–g*, formed successively after repeated elongation. *I*, Conidia, *a–s*, showing variations in size and shape. *J*, Germinating conidium, with two delicate lateral processes, probably functional as organs of capture. *K*, Sexual apparatus, showing origin of young zygosporangia, *a* and *b*, from conjugating branches arising from separate hyphae. *L*, Zygosporangium likewise resulting from union of branches arising from separate hyphae, but in somewhat later stage of development. *M*, Mature zygospore with enveloping zygosporangial membrane. In *G* and in *H* a dotted line indicates approximately the position of the surface of the substratum.

A NEW SPECIES OF CONIDIAL PHYCOMYCETE PREYING ON NEMATODES

Charles Drechsler

(with 1 text figure)

Although the Zoopagaceae hitherto observed in Petri dish cultures started from decaying plant materials consist preponderantly of forms destructive to *Amoebae,* at least several fungi undoubtedly referable to the same taxonomic group have been found that evidently subsist entirely by capturing and consuming nematodes. Of these several fungi the one whose morphology and predacious habit were briefly set forth in the text and synoptic illustrations of an earlier summary (**1**, *p. 140, lines 6 to 13; p. 139, fig. 8, A, C*) has made its appearance by far most frequently. In the vicinity of Washington, D. C., it seems to be present on leaf mold wherever in parks on other wooded tracts this material has had opportunity to accumulate in deposits deep enough to retain some moisture during periods of dry weather. When a pinch of leaf mold from such a deposit is added to an agar plate culture already well infested with nematodes, the fungus develops with considerable regularity, giving rise within 5 to 15 days to a growth, which, if ordinarily too scanty to be readily noticed with the naked eye, is fairly conspicuous under a microscope of low magnification.

MORPHOLOGY, DEVELOPMENT, AND DESCRIPTION

The rather meager mycelium thus revealed is composed of originally continuous hyphae approximately equal in width to the hyphae of the more familiar species of *Aphanomyces, Pythium* and *Phytophthora* occurring in diseased vegetable tissues (fig. 1, A–E). Variations in width are neither frequent nor pronounced, a branch being generally of about the same diameter as the parent filament, and maintaining this diameter without marked diminution well toward its growing tip. Branching occurs at irregular intervals and often at angles approaching a right angle, thus bringing about a characteristically stiff haphazard arrangement of the vege-

tative thallus. The living uninjured hyphae are filled with moderately and uniformly densely granular material, comparable in texture with the protoplasmic contents of the coarser species of *Pythium,* or, perhaps, intermediate in consistency between the protoplasmic material of the genus *Pythium* considered as a whole, and that of the genus *Phytophthora.* The older portions of the mycelium, as in many other filamentous Phycomycetes, undergo progressive evacuation, the retreating contents leaving behind thickish septa at intervals in the empty hyphal envelopes (FIG. 1, C, D). Similar evacuation and deposition of cross-walls takes place also in portions of younger hyphae that have become injured through the protracted and often very violent struggles of captured nematodes (FIG. 1, A).

Capture of prey is effected by means of a yellow adhesive substance similar in appearance to that secreted by the species of *Acaulopage* and *Stylopage* destructive to *Amoebae* (**5**). Operating in conjunction with this material are definitely differentiated structures in the form of largish globose protuberances (FIG. 1, A, *a, b, c*). Apparently these protuberances, unlike the stalked adhesive organs of *Dactylaria condida* (Nees) Sacc., are not formed beforehand to await the passage of suitable animals. As they have been found only where nematodes had already been caught, it would seem that their development follows rather than precedes contact with prey. As far as can be determined the animal is first held fast by a local deposit of a sticky substance secreted by the vegetative hypha at a place not otherwise markedly differentiated. In the course of time, as the animal struggles to free itself, there is thrust through the adhesive cake a lateral process, which, upon reaching the integument of the nematode, expands into the globose protuberance. Evidently the thick yellow wall of the protuberance is copiously covered over with adhesive material; so that with the extensive contact afforded by the expanded surface, the animal is held securely. Frequently two or more protuberances participate in catching a nematode (FIG. 1, A, *a, b*).

Although capture is thus accomplished altogether through adhesion without structural involvement, vigorous eelworms up to 0.5 mm. in length referable to such genera as *Rhabditis, Cephalobus, Diplogaster, Diploscapter, Acrobeles,* and *Acrobeloides,* are

held in spite of violent attempts at liberation. In contrast to most nematode-capturing Hyphomycetes, which in a few hours bring about the death of their prey by severing its organs either through intrusion of a bulbous outgrowth, or, more amazingly, through the strangulating action of constricting loops, the present fungus employs no special means of hastening the end of a captured animal. Extensive invasion is therefore of necessity delayed until the captive, after many hours of exertion, has become somewhat quiescent, in the main apparently from exhaustion. Eventually the animal's integument is perforated, and from the adhesive protuberance is intruded an outgrowth that immediately gives rise to hyphae which soon elongate and ramify to permeate the fleshy interior throughout (FIG. 1, A, *d*). The advance of the endozoic hyphae, which are about half as wide as the mycelial filaments, is everywhere reflected in visible degeneration of the organs and musculature of the eelworm. Gradually the degenerated contents become more and more attenuated, until finally they vanish completely. When this process of absorption is nearing completion, the protoplasm in the haustorial filaments begins to migrate back into the mycelial hypha, laying down rather widely spaced septa in its retreat (FIG. 1, B). As the evacuated haustorial system soon becomes largely if not wholly invisible, in the end only the empty and mostly collapsed integument of the nematode is to be seen adhering to the one or more protuberances, which are now walled off from the haustorial elements they had earlier put forth (FIG. 1, A, *e*).

Once a mycelium attains some size it gives rise to a scattering of tall erect conidiophores. These often conclude their development in producing individually a single large obovoid conidium (FIG. 1, C); but with more abundant nourishment they may continue growth from below the first conidium to produce a second farther on (FIG. 1, D, E), and sometimes after repeated elongation to a third, and occasionally even to a fourth. The conidia at maturity (FIG. 1, L–W) drop off on slight disturbance, and then usually without much delay germinate individually by the production of a stout hypha from the apex or from the zone immediately surrounding the basal hilum (FIG. 1, F–K). Despite the readiness with

which the conidia germinate, attempts at growing the fungus in pure culture on maizemeal agar have not been successful.

The development of the asexual reproductive apparatus manifestly reveals a close correspondence with the homologous phase in the development of the three known amoeba-capturing species of *Stylopage*. Because of the frequent production of more than one conidium on a single conidiophore the parallelism with *S. lepte* Drechsl. is especially complete. The great disparity in dimensions might at first seem to compel interpretation of this close parallelism as being fortuitous. However, the differences in size appear far from impossible to reconcile with close relationship of the two fungi, after consideration of *S. araea* Drechsl. In the latter species are expressed, on the one hand, unquestionable similarities in general dimensions and predacious relationships to *S. lepte;* and, on the other, obvious even if only partial approximation to the nematode-capturing form with respect to stature of conidiophore as well as to size and shape of conidium. The fungus predacious on nematodes is therefore assigned with reasonable assurance to the genus *Stylopage*. A term having reference to its robust stature is deemed appropriate as specific name.

Stylopage hadra sp. nov.

Sparsa; hyphis sterilibus incoloratis, 3.5–5.5 μ crassis, tubera orbicularia flavida glutinosa usque 15 μ lata et longa evolventibus; his tuberibus animalia tenentibus, integumentum perforantibus, hyphas 2–2.5 μ crassas intus evolventibus, carnem exhaurientibus. Hyphae fertiles 200–400 μ altae, basi 4–5.5 crassae, sursum attenuatae, apice 2–2.5 μ crassae, unicum conidium vel interdum usque 3–4 conidia post incrementa repetita ferentes; conidiis incoloratis, obovoideis, 20–45 μ longis, 13–23 latis. Zygosporae ignotae.

Habitat in terra, in materiis plantarum putrescentibus, praecipue in humo silvarum, nematoda diversa usque .5 mm. longa capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, 3.5 to 5.5 μ wide, forming yellow adhesive orbicular protuberances up to 15 μ in diameter, by means of these protuberances holding nematodes, perforating the integument of each, inside producing hyphae 2 to 2.5 μ wide and assimilating the fleshy contents. Conidiophore 200 to 400 μ high, 4 to 5.5 μ wide at the base, tapering upward, 2 to 2.5 μ wide at the tip, bearing a single conidium, or often producing up to 3 or 4 conidia one by one after repeated elongation. Conidia colorless, obovoid, 20 to 45 μ long and 13 to 23 μ wide. Zygospores unknown.

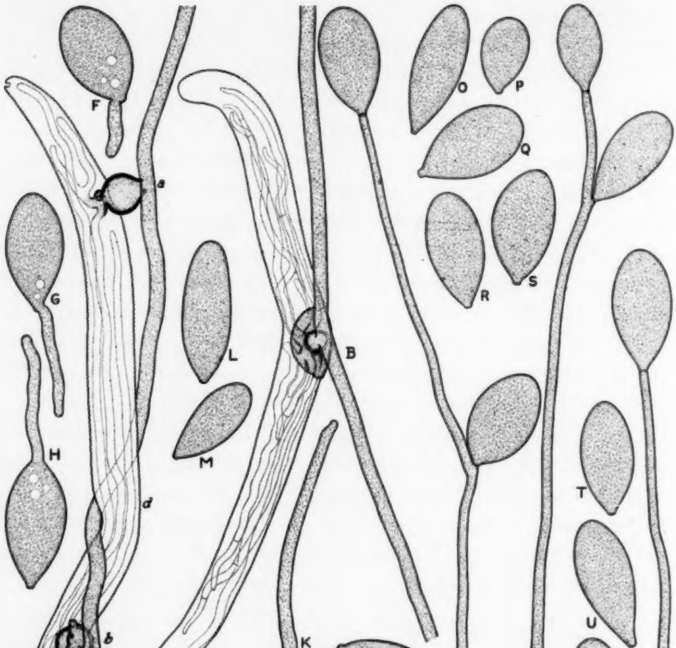

FIG. 1. *Stylopage hadra.*

Occurring in soil, in decaying plant materials but especially abundantly in leaf mold; capturing and consuming nematodes up to .5 mm. long belonging to various species of *Rhabditis, Cephalobus, Diploscapter, Diplogaster, Acrobeles* and *Acrobeloides*, near Washington, D. C.

TAXONOMIC CONSIDERATIONS

The species is apparently not the only representative of its group subsisting on nematodes. Similarities in character of vegetative mycelium and in mode of capture give reason to believe that the predacious fungus with *Pythium*-like intercalary chlamydospores figured earlier (**2**, *fig. 15, D, C*) may be closely related to it. A fungus not hitherto referred to, which likewise captures nematodes through adhesion to a continuous mycelium, and which on a short prolongation from the union of two branches coming from separate hyphae gives rise to a zygospore about 15 μ in diameter within a closely fitting zygosporangial wall irregularly sculptured with yellow incrustation, undoubtedly represents another member of the group. From these two species, which it is hoped may be more fully discussed after their asexual stages are more completely known, *Stylopage hadra* differs in the moderate and sometimes even rather meager development of its mycelium. This inextensive development finds a plausible ecological explanation in the evident adaptation of the fungus for the capture of the larger and correspondingly more vigorous nematodes. The brisk locomotor movements of these animals insures, on artificial substrata, and presumably also in nature, adequate encounter with prey notwithstanding the moderate extension of the predacious apparatus. Once a relatively powerful animal has been engaged, however, physical sturdiness is required both to hold it securely and to endure the inevitable violence without incurring too severe injury. For although the predacious Hyphomycetes suffer little damage when their organs of capture, together often with connected portions of mycelium, are uprooted, a phycomycete would obviously be more seriously affected if portions of its non-septate thallus were constantly being torn away. Indeed, in spite of the considerable measure of sturdiness attained at the expense of a wider extension, local damage is very frequently plainly evident.

The somewhat inextensive mycelial development, whatever its explanation, brings about an appearance vaguely suggestive of some members of the Entomophthorales. This suggestiveness is sustained in the large size of the conidia, and their similarity in shape to the ovoid conidia described and figured by Thaxter (**11**) in the presentations more particularly of his *Empusa americana, E. montana,* and *E. echinospora.* Occasionally, too, the hypha of germination from a conidium gives rise to a second conidium with so little intervention of a purely vegetative phase that the repetitional development of secondary conidia frequent in many species of the Entomophthorales is approximated. As such repetitional development is fairly widespread among various groups of fungi, occurring for example, in conspicuous measure in many of the predacious hyphomycetous forms referable to *Monacrosporium* and *Dactylaria,* its importance as an indication of affinity hardly merits emphasis. Yet in the absence of all intimate parallelism with any other of the older established groups within the Zygomycetes, the suggestive correspondencies with the insectivorous Entomophthorales, among which a semi-predacious habit of fixing their enfeebled prey to the substratum by means of adhesive substance is frequent, are at least deserving of mention.

Its frequent occurrence in leaf mold and in similar nematode-infested decaying materials, together with the large dimensions of its conidia and conidiophores, would make it seem unlikely that *Stylopage hadra* could have remained unobserved by the numerous mycologists that have devoted themselves to the study of fungi appearing on animal refuse and on decomposing plant remains. Once observed, it might be supposed that the fungus would almost certainly have evoked more than ordinary interest by virtue of morphological features, which, while obviously pertaining to a member of the Phycomycetes, do not conform to those of any of the groups long recognized in that class. That such interest failed to develop may perhaps be attributed less to the fungus having been overlooked than to its probably having been confused with nematode-capturing Hyphomycetes, and of these more particularly with two forms with which it appears in almost habitual association: the fungus with swollen 3-septate conidia and constricting loops figured previously (**3**, *fig. 17, A, C*), and possibly

to be identified as *Monacrosporium elegans* Oud. (**8**); and the fungus with somewhat fusiform 4-septate conidia and stalked adhesive knob-cells (**1**, *fig. 7, A, B, C*) corresponding well to Grove's (**6**) description of his *Dactylella ellipsospora* (**4**). Very curiously, whether through morphological accident, or, more probably, through what would seem to constitute a remarkable instance of convergence resulting from similarity in predacious relationship, the two Hyphomycetes mentioned show approximate similarity to *S. hadra* in the dimensions and erect posture of their conidiophores, as well as in the dimensions and shape of their conidia. Their conidiophores, moreover, like the homologous structures of predacious Hyphomycetes generally, show few septa, and often do not develop these until a relatively late stage. On closer inspection of material in agar cultures, the presence of numerous cross-walls dividing adjacent living cells in the mycelial filaments, and the nearly homogeneous consistency of the protoplasm surrounding the well defined largish vacuoles, are easily recognized as features alien to the fungus under consideration. But when the vegetative mycelium is concealed in an opaque natural substratum, the similarities in habit of the erect aerial parts are brought into deceptively strong relief; so that the conidial apparatus of the Phycomycete might then readily be mistaken for immature apparatus of either of the two Hyphomycetes often accompanying it.

In 1851 Preuss (**9**) described and figured under the binomial *Menispora ellipsospora* a fungus he found on decaying needles of Scotch fir where it formed thinly effuse growths consisting of white erect non-septate conidiophores bearing individually a single terminal large elliptical spore. According to the description a large oil globule occupied the entire lumen in the median portion of the conidium, which thus came to reveal toward each of its ends a curved contour extending entirely across its width. No mention was made of septa occurring in the conidia of either this species or of *Menispora pyriformis*, which Preuss described at the same time; nor were such septa shown in any of the accompanying figures. The non-septate condition ascribed to the conidia of *Menispora ellipsospora* and *Menispora pyriformis* was emphasized by Oudemans (**8**) in distinguishing his *Monacrosporium elegans*

from these species despite the similarity in habit clearly recognized by him. Grove (**6**) on the other hand considered *Menispora ellipsospora* identical with his *Dactylella ellipsospora,* and therefore cited Preuss' binomial as a synonym. Later Saccardo (**10**, *p. 194*) transferred also *Menispora pyriformis* to the genus *Dactylella.* Lindau (**7**, *p. 411–412*), though adopting the transfers thus made, commented on the uncertain status of *D. pyriformis,* stating that not even the condition of the conidia, whether continuous or septate, was definitely known.

Since in Preuss' account of *Menispora pyriformis,* at least the conidiophores were described as sometimes containing septa, Lindau's doubts might with even more justification have been directed at *Menispora ellipsospora.* Certainly in its main features the original description of the latter fits *Stylopage hadra* better than *Dactylella ellipsospora* or, for that matter, than any similarly septate hyphomycetous form. However, vacuoles of any considerable size are not usually discerned in the conidia of *S. hadra,* nor in those of the three amoeba-capturing species of *Stylopage;* whereas, very large vacuoles regularly are found in the inflated median cells in the well matured conidia of various predacious Hyphomycetes. In any case the vacuolate condition figured by Preuss, which would perhaps need to be considered rather extreme even for a species of *Trichothecium, Monacrosporium,* or *Dactylaria,* appears very definitely foreign to the nematode-capturing phycomycete herein described. This difference in the internal structure of the conidium precludes identification of the fungus with *Menispora ellipsospora* hardly less decisively than the presumptive difference in condition of the conidiophore relative to septation precludes identification with *Menispora pyriformis.* Apart from the two binomials mentioned, the established application of the genus *Menispora* to a distinctive group in the Dematiaceae obviates the possibility of further nomenclatorial or taxonomic involvement with *Stylopage.*

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EXPLANATION OF FIGURE

Fig. 1. *Stylopage hadra;* drawn with aid of camera lucida at a uniform magnification; × 500. *A*, Portion of hypha on which have been developed three adhesive protuberances, *a–c*; two of which, *a* and *b*, have been operative in the capture and invasion of a rather large nematode, *d*, referable apparently to *Acrobeloides Bütschlii* (De Man, 1885) Thorne, 1925; and the third, *c*, has captured a small nematode evidently of the same species, depleted its contents, and withdrawn the protoplasm from the haustorial elements by means of which the depletion was accomplished. *B*, Portion of hypha with an adhesive protuberance, on which has been captured a nematode belonging to *Cephalobus* sp.; the eelworm is thoroughly permeated with haustorial hyphae, from which, following depletion of the fleshy tissues, the protoplasmic contents are being withdrawn, as is indicated by the appearance of septa near the hyphal ends. *C*, Portion of prostrate hypha bearing a relatively short conidiophore which has given rise to a single conidium. *D*, Portion of mycelium, and arising from it, a conidiophore whereon two conidia have been produced successively; owing to its length the conidiophore is shown in sections, on which *a* and *b* represent corresponding points. *E*, Portion of mycelium from which arises a conidiophore bearing two conidia. *F–K*, Germinating conidia. *L–W*, Conidia showing variations in size and shape.

A NEW MUCEDINACEOUS FUNGUS CAPTURING AND CONSUMING AMOEBA VERRUCOSA

Charles Drechsler

(with 1 text figure)

In an earlier summary setting forth the morphological features of some fungi that had been found capturing and consuming *Amoebae* in agar plate cultures started from plantings of diseased rootlets and other decaying vegetable materials, was included a brief characterization (**3**, *p. 200, lines 19 to 34; p. 201, fig. 1, A, B*) of a septate species predacious on an *Amoeba* then provisionally determined as *Amoeba verrucosa* Ehrenb. The determination can advantageously be retained, since the protozoan, in its relatively large dimensions, its single ellipsoidal nucleus, its slow movement, and its extraordinarily thick pellicle, agrees well with Leidy's description (**10**) of *A. verrucosa*. Though the animal might also be referred to *A. terricola* Ehrenb. in the broad sense in which that species was understood by Penard (**14**), it is apparently not identical with any one of the three separate forms to which I have elsewhere (**6**) applied this binomial together with the numerals I, II, and III, respectively. Of these three forms, it most nearly resembles the one designated as *A. terricola* II, being distinguished therefrom, however, by a different distribution of dark material within the nucleus, and by a much greater thickness of the pellicle (fig. 1, A). It has been found to develop rather rarely on plate cultures, probably requiring conditions for multiplication not often provided by agar substrata. Owing apparently to this infrequent development, the septate fungus that lives, as far as has been observed, entirely by the capture of the protozoan in question, has put in appearance only a few times.

The mycelium on a transparent substratum like maizemeal agar is similar in general aspect to the mycelium of *Pedilospora dactylopaga* Drechsl., a mucedinaceous fungus known to subsist on shelled rhizopods (**5**). It reveals a similar sparsely effuse habit

with approximately equally meager branching. The hyphae, which follow rather straightforward courses, in large part on the surface of the substratum, while somewhat wider than the hyphae of *P. dactylopaga*, contain like these, cross-walls separating adjacent living cells, which are filled, except for occasional vacuoles, with protoplasm of fairly homogeneous consistency. At irregular intervals on the hyphae are borne prolate ellipsoidal protuberances that although slightly longer and noticeably more obese obviously correspond to the digitate or elongate-elliptical protuberances of *P. dactylopaga* both in morphology and in function.

An animal on coming in contact with one of the protuberances remains adhering to it, evidently being held by means of glutinous material. Whether the captive makes any effort to escape, apart from movements normally entailed in locomotion, remains uncertain. In any case the substantial pellicle of the animal is perforated when the protuberance puts forth a filamentous outgrowth that penetrates deeply into the protoplasmic interior, at the same time widening gradually in its course. On attaining definitive length, the outgrowth branches dichotomously; the resulting elements very soon bifurcating again (FIG. 1, B), often in planes at right angles to the primary dichotomy (FIG. 1, A). Repeated dichotomous branching follows until the central portion of the animal is occupied by a rather elaborately ramifying apparatus (**3**, *fig. 1, B*). This apparatus at first is continuous but later becomes divided by septa into a number of variously shaped segments (FIG. 1, C, *a*). From these segments, on the depletion of the animal's protoplasmic materials, are put forth narrow hyphae that pass out through the pellicle to extend the predacious mycelium or to give rise to conidiophores and conidia. The pellicle eventually collapses, and persists long as a wrinkled mass testifying to the destructive efficacy of the fungus.

Usually after a few animals have been consumed, conidiophores are produced in small groups scattered here and there on the substratum (FIG. 1, D; **3**, *fig. 1, A*). Relatively short and sparingly branched, they present an atrophied appearance little reminiscent of the stately conidiophores characteristic of most of the nematode-capturing species of *Trichothecium, Arthrobotrys, Dactylella, Monacrosporium* and *Dactylaria.* And the narrow conidia (FIG.

1, D, *a*; E, *a–e*; **3**, *fig. 1, A*) borne on these meager reproductive hyphae are correspondingly little suggestive of the conidia produced by the more robust of the hyphomycetous forms preying habitually on eelworms. The empty distal appendage present on the conidium finds no homologue among any of the other Hyphomycetes now known to be predacious, providing instead a striking parallelism with some *Amoeba*-capturing Phycomycetes described elsewhere (**7**) as members of the genus *Acaulopage*.

Yet the dissimilarities in outward form just noted are hardly such as to preclude a fairly close taxonomic relationship. In the group of *Amoeba*-capturing Phycomycetes, species with well developed conidial appendages are most obviously closely connected with species having only rudimentary appendages, and even with species altogether devoid of such modifications. If the conidiophores of the fungus under consideration are unimpressive in comparison with those of *Pedilospora dactylopaga,* they would seem, judging from Höhnel's original account (**9**), quite comparable with the conidiophores of *P. parasitans,* a form whose intimate relationship to *P. dactylopaga* cannot readily be questioned. The thoroughgoing resemblance to the latter species with respect to mycelial characters, may therefore be presumed with a fair degree of certainty to indicate membership in the group of closely interrelated predacious Hyphomycetes most familiarly exemplified in *Arthrobotrys oligospora* Fres.

In considering an appropriate disposition of the fungus, this presumptive relationship deserves to be taken into account. If the conidium is regarded as being composed of two cells, it would be difficult to avoid assignment to *Trichothecium,* of which genus three established species, *T. obovatum* (Berk.) Sacc., *T. piriferum* (Fries) Sacc., and *T. inaequale* Mass. & Salm., would seem from their resemblance in habitat, habit, and morphology to the nematode-capturing form figured earlier (**2**, *fig. 10, A, C*), to represent members of the same predacious series. However, with respect to shape of conidium and to position of the septum within the conidium, these species diverge markedly from the one under discussion. Assignment to *Trichothecium* is further discouraged from the fact that this genus has in large part become familiar to mycologists generally through *T. roseum* Link, a widespread sapro-

phyte and plant parasite that has revealed no predacious tendencies whatever under experimental conditions, and that in morphology is plainly alien to the predacious series.

A more apt disposition in the genus *Dactylella* or *Monacrosporium* is feasible if, as seems permissible, the empty distal conidial appendage is construed as a third cell. Both these genera were erected on species that may rather safely be presumed to belong to the predacious series: *D. minuta* Grove (**8**) presenting strong similarities in habitat, habit, and morphology to known nematode-capturing forms; while the description of *M. elegans* Oud. (**13**) except for a somewhat greater length of conidium, applies very well to one of the most abundant and widespread of nematode-capturing forms figured earlier (**4**, *fig. 17, A, C*). Like the three established species of *Trichothecium* mentioned, these broad-spored type-species show little family resemblance to the *Amoeba*-capturing fungus; nor is such resemblance greatly evident in the broad-spored nematode-capturing *D. ellipsospora* Grove (= *M. leporinum* Bubák), or in the similarly broad-spored and presumably similarly predacious *D. rhombospora* Grove and *M. ovatum* Petch (**15**). A closer approximation in general make-up is apparent in the allied forms with narrower conidia, including more particularly *D. minuta* var. *fusiformis* Grove, *M. subtile* Oud. *M. oxysporum* Sacc. & March., and *M. sarcopodioides* (Harz) Berl. & Vogl., which from their resemblance in habitat, habit and morphology to the somewhat *Fusarium*-like nematode-capturing fungus figured earlier (**4**, *fig. 16, A–C*) must be reckoned among the presumptive members of the predacious series.

Saccardo (**17**, *p. 193*) early recognized the affinity between *Dactylella* and *Monacrosporium*, but nevertheless held the latter genus distinct from the former because of the presence of copious mycelium. Lindau (**11**, p. *412*) properly regarded the distinction based on the presence of copious mycelium as in itself insignificant, yet adopted it in the belief that whereas the species of *Monacrosporium* probably constitute conidial stages of the coprophilous Sordariaceae, *Dactylella* might more likely be referable to other Pyrenomycetes. An understanding of the predacious habits of the fungi in question places in a different light the substratum relationships on which Lindau's tentative assumption of divergent

pleomorphic connections must have been founded. Moreover, since in pure cultures of such of the predacious forms under discussion as have been isolated, the relative abundance of aerial mycelium is often hardly of sufficient distinctiveness to merit attention in the separation of species, its utility for the separation of genera seems exceedingly doubtful.

The equivalence of the two genera was disturbed more recently when Boedijn (**1**) extended the application of *Monacrosporium* by describing under the name *M. megasporum* a fungus producing conidia somewhat similar to those of *M. elegans*, but bearing them in closely capitate arrangement. However, as the fungus, evidently an authentic member of the predacious series, answers exactly to the definition of the genus *Dactylaria*, within which it would have found at least one closely related predacious congener in *D. candida* (Nees) Sacc., and perhaps others in *D. acicularis* Rostrup (**16**) and *D. pulchra* Linder (**12**), the extension seems hardly possible of adoption.

In assigning the *Amoeba*-capturing fungus, considerations of priority dictate a preference for *Dactylella*, erected in 1884, over *Monacrosporium*, proposed in 1885 (not apparently in 1884 as is often stated). A term having reference to the knoblike organs of capture is deemed appropriate as specific name.

Dactylella tylopaga sp. nov.

Mycelium sparsum, repens, parce ramosum; hyphis sterilibus 1.5–3 μ crassis, hyalinis, mediocriter septatis, hinc inde tubera ovoidea vel ellipsoidea 4–7.5 μ longa, 3.5–5.5 μ crassa, verisimiliter glutinosa, primo hyalina mox flavida emittentibus, his tuberibus animalia capientibus, pelliculam perforantibus, ramum intus evolventibus; ramo primo hyalino, mox saepe flavente, ad centrum animalium penetrante, sursum paulatim latescente, ramos 2.5–6 μ crassos repetite dichotomos mox septatos gerente; his ramis protoplasma consumentibus, hyphas mycelii extus evolventibus. Hyphae fertiles paucae, hyalinae, assurgentes, saepe plus minusve ramosae, 15–50 μ altae, basi 3–5 μ crassae, sursum attenuatae, apice 1–1.3 μ crassae, conidia singulatim gerentes; conidiis hyalinis, in totum 30–50 μ longis, parte supera eorundem vacua itaque appendicula marcida 13–23 μ longa, basi 1–1.5 μ lata, sursum attenuata, apice .5–1 μ lata facta; parte infera in cellulas duas subaequales, protoplasmatis repletas, 9–17 μ longas, 2.5–3.5 μ latas, divisa.

Habitat in humo silvarum *Amoebam verrucosam* capiens et consumens prope Washington, D. C.

Mycelium sparse, creeping, meagerly branched; vegetative hyphae hyaline, 1.5 to 3 μ wide, moderately septate, at intervals bear-

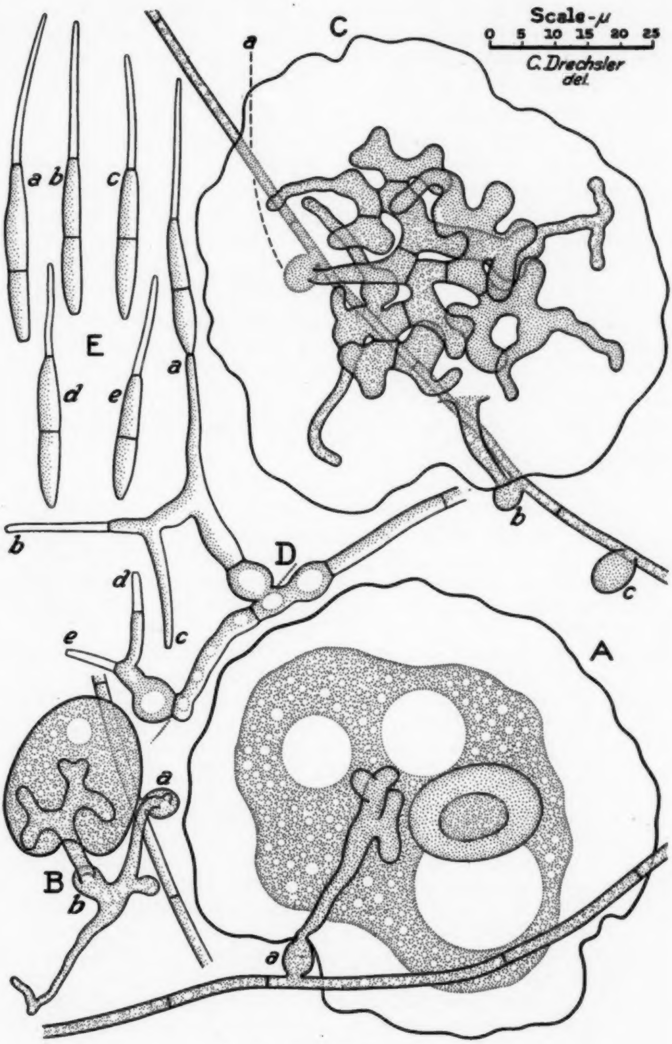

FIG. 1. *Dactylella tylopaga.*

ing ovoid or ellipsoid, apparently adhesive, and ultimately yellowish protuberances, 4 to 7.5 μ long and 3.5 to 5.5 μ wide; by means of these protuberances capturing animals, perforating the pellicle of each and developing a branch inside; the branch at first hyaline, often turning yellowish after penetrating toward the center of the animal while widening in its course, then giving rise to repeatedly dichotomous branches 2.5–6 μ wide, which, though originally continuous, after consuming the animal's protoplasm finally become septate and emit vegetative filaments. Conidiophores few, hyaline, ascending, often more or less branched, 15 to 50 μ high, individually 3 to 5 μ wide at the base, tapering upward, 1 to 1.3 μ wide at the tip. Conidia borne singly, 30 to 50 μ in total length, the upper part of each empty and accordingly present as a withered appendage 13 to 23 μ long, 1 to 1.5 μ wide at the base, tapering upward to an apical width of .5 to 1 μ; the lower part divided into two subequal cells filled with protoplasm, each 9 to 17 μ long and 2.5 to 3.5 μ wide.

Occurring in leaf mold, capturing and consuming *Amoeba verrucosa* near Washington, D. C.

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EXPLANATION OF FIGURE

Fig. 1. *Dactylella tylopaga* drawn with aid of camera lucida at a uniform magnification; × 1000. *A*, Portion of hypha with an adhesive protuberance, *a*, from which a widening branch has been intruded into a captured specimen of *Amoeba verrucosa*. *B*, Portion of hypha with an adhesive protuberance, *a*, that has proliferated an irregular outgrowth bearing a second protuberance, *b*, from which a dichotomously branching process has been thrust into a captured specimen of *A. verrucosa*. *C*, Portion of hypha with three adhesive protuberances, *a–c*, whereof two, *a* and *b*, have captured a specimen of *A. verrucosa*, invaded it extensively, and completely consumed its contents, leaving only the thick empty pellicle; septa have been inserted in the dichotomously branching system, and some of the delimited segments have begun to proliferate ordinary hyphae. (For the sake of clearness the branching development from protuberance *b* is omitted.) *D*, Superficial hypha with two conidiophores, one with three branches, *a–c*, the other with two branches, *a* and *b*; branch *a* being filled with protoplasm and bearing a mature conidium; branches *b*, *d*, and *e* having been partly evacuated. *E*, Mature conidia, *a–e*, showing variations in size and shape.

NOTES AND BRIEF ARTICLES

Key to Symbols Used by Berkeley and Curtis in Their Copies of Schweinitz' "Synopsis Fungorum in America Boreali"

It is the good fortune of American mycologists that there are available in connection with two large collections of fungi in this country the identical copies of Schweinitz' "Synopsis Fungorum in America Boreali" used and annotated by Berkeley and by Curtis when they were studying Schweinitz' specimens. Berkeley's copy is in the Library of the U. S. Department of Agriculture at Washington, and Curtis' copy is in the Farlow Herbarium at Harvard. It is believed that an explanation of the symbols they used in checking the species will be of interest and value to those who may consult these volumes.

Some years ago the writers called attention in this journal (**9**: 338) to the apparent significance of the symbols used by Berkeley before the species in his copy which are as follows:

H—Specimen found in the Hooker collection at Kew.
C—Specimen loaned him by Curtis.
V—Specimen from Curtis in Berkeley's own herbarium at Kew.

Since this was published, the writers have had opportunity to study Curtis' personal copy of Schweinitz and have made the following key to the symbols used by Curtis:

+ Indicates any Schweinitz specimens he examined.
! + Indicates that part of the specimen was taken by Curtis and loaned to Berkeley. This was to be returned and presumably is to be found in Curtis' Herbarium.
O ! + Indicates part of the specimen was taken and divided with Berkeley and is now in his Herbarium at Kew.

The following equivalence is thus evident:

C Berkeley = ! + of Curtis and means that a specimen should be in the Curtis collection at Harvard.

V Berkeley (in large part = O ! + of Curtis and means that specimens are in both the Berkeley herbarium at Kew and the Curtis' herbarium at Harvard.

Comparison of the two copies checked with numerous specimens in Curtis' herbarium at Harvard and the collection at Kew gives overwhelming evidence as to the correctness of the above interpretation of these keys.

C. L. SHEAR AND N. E. STEVENS

THE MYCOLOGICAL SOCIETY OF AMERICA

REPORT OF THE THIRD ANNUAL MEETING

The third annual mid-winter meeting of the Mycological Society of America was held December 27, 28, and 29 at Pittsburgh, Pennsylvania, in conjunction with that of the American Association for the Advancement of Science. The Society had been granted formal affiliation with the Association during the year, and was represented on the Association Council by our two past presidents, Wm. H. Weston, Jr. and C. L. Shear. The headquarters of the Society were at the William Penn Hotel, one of the largest and finest hotels in the State. This hotel served also as headquarters for the Botanical Society of American, American Phytopathological Society, and other botanical groups. Unusually ample and excellent facilities were afforded for conferences and informal get-togethers among the many botanists thus brought together. The sessions of the botanical societies were held for the most part in the Cathedral of Learning of the University of Pittsburgh, a very tall and exceptionally beautiful building. The arrangements made for the Mycological Society by the local representative, Doctor Otto E. Jennings, were most satisfactory. Favorable weather added to the pleasure of the meeting.

The retiring president, H. S. Jackson, presided at the sessions of the Society, and gave as his address an account of his recent researches on some interesting Heterobasidiomycetes on ferns. The Society held the usual joint sessions with Section G and the American Phytopathological Society. Saturday afternoon was set

aside for the giving of demonstrations of research materials, discussed in the earlier sessions. Interesting displays were made by C. L. Porter of fungi found in apparently fossil condition in Australian sands, by Morris Moore of species parasitic in man and animals, and by S. M. Pady of intracellular mycelium in *Gymnoconia*.

At the regular sessions about thirty mycological papers were presented. Though they dealt with many groups of fungi and many phases of mycology, contributions on cytological and developmental studies were perhaps most outstanding.

At the business session on Thursday morning a committee was named by the president to draft expressions of regret at the passing of several members during 1934. Those removed by death during the year were Charles Fairman, Mrs. Esther Lewis, Thomas H. Macbride, and Frank L. Stevens. New officers elected for 1935 are Bernard O. Dodge, president, John Dearness, vice-president, and Cornelius L. Shear, councilor. The Council named John A. Stevenson to serve an additional five-year term as associate editor of MYCOLOGIA. The editor-in-chief Fred J. Seaver reported on the financial condition of the journal, and announced a plan whereby members may obtain back volumes of MYCOLOGIA in exchange for herbarium specimens. Those interested should write to him. The report of the secretary-treasurer shows the finances of the Society to be in good condition. All members are urged to invite graduate students and others to join as associates if regular membership constitutes too heavy an obligation. Associate members do not receive Mycologia and may not vote, but they have essentially all the other privileges of regular members including the right to appear on the program. The roll of the Society now includes 338 names, and the membership is slowly growing. The year book for 1935 is now in press and will be mailed early in February.

In response to an invitation from Doctor M. J. Sirks, Honorary Secretary of the Organizing Committee for the Sixth International Botanical Congress, the Council has selected David H. Linder, Fred J. Seaver, and Cornelius L. Shear as delegates to represent the Society in Amsterdam next September.

H. M. FITZPATRICK, *Secretary-Treasurer*

THE GENUS ZYGOSPERMUM

In a recent publication, "Studies of Coprophilous Sphaeriales in Ontario," Univ. Toronto Studies, Biol. Ser. **38**: 73. 1934, the author proposed the name *Zygospermum* for a new genus of Sphaeriales. It has come to my attention that this name has been previously used as a genus by Baillon.[1] Apparently no one except Baillon has taken it up as a genus and he himself reduced it to synonymy a few years later. Nevertheless the name becomes invalid in the sense in which I have used it.

The following new generic name is therefore proposed.

Zygospermella gen. nov.
Zygospermum Cain, l.c. p. 73.
Zygospermella setosa comb. nov.
Zygospermum setosum Cain, l.c. p. 74.
Zygospermella insignis (Mout.) comb. nov.
Zygospermum insigne (Mout.) Cain, l.c. p. 76.

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A CORRECTION

Since the publication of the name *Pholiota intermedia* Singer for another species (Beih. Bot. Centr. Abt. **46**^{2}: 107. 1929), antedates the publication of *Pholiota intermedia* Smith (Ann. Myc. **32**: 479. 1934) it is necessary to give a new name to the latter fungus. **Pholiota septentrionalis** nom. nov. is proposed.—ALEXANDER H. SMITH.

[1] *Zygospermum* Thwaites ex Baillon, Étud. gén. Euphorb. 620, t. 27. 1858.